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CAUSES OF COLOR CHANGE IN BLUE-GREEN ALGAE

By Marston C. Sargent

William G. Kerckhoff Laboratories of the Biological Sciences,
California Institute of Technology, Pasadena, California

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The characteristic colors of the different classes of algae and the striking changes in color of individuals (especially among the Cyanophyceae) have compelled the attention of botanists for many years. Engelmann (1883) observed the rate of assimilation of a number of kinds of algae in the different parts of the spectrum. He concluded that they assimilated fastest in light of a color complementary to their own, and suggested that this effect controlled their distribution in nature. Gaidukov (1902) cultivated Cyanophyceae in light from different parts of the spectrum and found that they assumed a color complementary to that of the incident light. This phenomenon he called "complementary chromatic adaptation." However, Oltmanns (1893) grew marine algae in light of different colors and light of different intensities and concluded that the differences in color of the algae depended principally on differences in intensity. A third proposal was made by Schindler (1913) on the basis of experiments on the cultivation of Oscillatoriaceae. He decided that the color of the algae depended primarily on the supply of essential food materials, especially nitrogen compounds, and that the intensity of light influenced only the *rate* of color change by influencing the rate of the organisms' growth and hence of exhaustion of the medium. A large amount of work has appeared supporting each of the three doctrines. The best discussions of the literature are to be found in Schindler (1913) and Harder (1923).

Much of the work done hitherto has been accomplished under unfavorable circumstances. The organisms used have grown slowly or not at all or have died during the experiments (Harder 1922). The color changes have often been slight or have affected only some of the individuals studied (Gaidukov 1902). The experimenters have usually been satisfied to work at low light intensities or in the varying and interrupted light of day (v. Richter 1912, Boresch 1921, Harder 1923). The experiments of which a preliminary report is given below were conducted in the course of develop-

ing a standard technique of cultivation which would produce uniform material for work on the photosynthesis of a blue-green alga. The organism used develops vigorously and uniformly under the conditions devised, but if the illumination is varied in the ways described, it exhibits striking color changes. In my opinion the relation of the color changes to changes in the illumination is so clear that the conflicting theories of the past may be judged in its light.

The organism used in these experiments is *Gloeocapsa montana*, obtained in species-pure culture by the usual streaking and plating methods. It is cultivated essentially in the way described by Warburg (1922, p. 250), under sterile conditions, in an inorganic liquid medium through which a stream of gas is constantly passed. The culture flasks stand in a glass-bottomed water bath at about 20°C. illuminated from below by an incandescent bulb or glow-tube. Under these circumstances, the number of cells can double in three days and increase fifteen-fold in three weeks. Although bacteria are present, they are never sufficiently numerous to cloud the medium.

When the cells were grown at a distance of 25 cm. from a 40-watt incandescent bulb they were dark blue-green in color. When they were placed 10 cm. from a 100-watt bulb they became buff-colored in about ten days. When they were replaced in the less intense light they became dark blue-green in 48 hours. The cycle could be repeated several times with a single culture.

In order to distinguish between the possible effects of color and intensity of light, several sources of illumination in addition to incandescent bulbs were used. A mercury glow-tube operated by a 15,000-volt 30-milliampere neon sign transformer furnished low intensity blue light. A hot-cathode mercury glow-tube operated by a 600-volt 1-ampere transformer furnished high intensity blue light. A neon glow-tube operated by the 15,000-volt transformer furnished high intensity red light, and a duplicate tube, screened by several layers of filter-paper, furnished low intensity red light.* In every experiment, the color change was conspicuous at a glance. No question arises about the relation of the color to the density of a culture, as masses of centrifuged cells always had the same color as the original suspensions. Parallel experiments gave the same results in all cases. In low intensity light whether white, blue or red, the cells became dark blue-green; in high intensity light they became yellow, or light green. The experiments were always ended long before the greatest possible growth had occurred so that the medium never approached exhaustion. In the absence of a satisfactory standard color-scale, a verbal description of the colors obtained must suffice.

It would be interesting to know the exact changes in amount of pigments taking place in the cells. Unfortunately, no method of making

extracts of the blue pigment, phycocyanin, so far devised, has been found satisfactory. A determination of the chlorophyll content was made at the end of each experiment. A measured volume of cells was extracted completely with methyl alcohol; the extract was made up to a standard volume and the extinction coefficient determined spectrophotometrically in light of wave-length 659 mμ from a neon tube. The table below gives the results, expressed as moles of chlorophyll in 1 c. mm. of cells (Emerson and Arnold 1932). The dry weight of 1 c. mm. of cells is about 0.03 mg.

ILLUMINATION		MOLES CHLOROPHYLL IN 1 MM.³ CELLS	
White	High	0.656×10^{-10}	
	Low	2.76×10^{-10}	
Blue	High	1.14×10^{-10}	1.28×10^{-10}
	Low	1.90×10^{-10}	2.06×10^{-10}
Red	High	0.676×10^{-10}	
	Low	1.73×10^{-10}	

It is evident that in every case there is less chlorophyll in the cells grown in the brighter light. The order of magnitude of the experimental error is indicated by the figures for two separate experiments in blue light.

To determine the effect of temperature, cultures were grown at 20° and 30° in low intensity blue light. No difference in color was apparent. Cultures have since been grown at 40° with no evident change. A slight difference in chlorophyll concentration was found, probably lying outside the experimental error.

TEMPERATURE	MOLES CHLOROPHYLL IN 1 MM.³ CELLS
30°	2.49×10^{-10}
20°	2.06×10^{-10}

In order to test the effect of changes in the medium, cultures were made up containing different amounts of iron. In general, the effect of lowering the amount of iron was the same as that of increasing the light intensity. The color changed in the low intensity blue light from blue-green to lime-green and in the high intensity blue light from gray-green to yellow. The changes in concentration of chlorophyll paralleled the changes in color.

EXPERIMENTAL CONDITIONS	MOLES CHLOROPHYLL IN 1 MM.³ CELLS	
High intensity white light	Fe 2.8 mg./l.	0.446×10^{-10}
	Fe trace	0.0744×10^{-10}
High intensity blue light	Fe 2.8 mg./l.	1.28×10^{-10}
	Fe trace	0.194×10^{-10}

From these observations it is evident that the color of *Gloeocapsa montana* is dependent primarily on the intensity of the light in which it is growing and on the composition of the medium. The experiments give no reason for excluding the possibility that color of incident light, and temperature, may have an effect on the color of the organism, but this effect must

be a minor one. In *Gloeocapsa montana* there occurs nothing of the nature of complementary chromatic adaptation.

* The glow-tubes were furnished through the courtesy of the Electrical Products Corporation, Los Angeles.

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ON SUPER-NOVAE

By W. Baade and F. Zwicky

Mount Wilson Observatory, Carnegie Institution of Washington and California Institute of Technology, Pasadena

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A. Common Novae.—The extensive investigations of extragalactic systems during recent years have brought to light the remarkable fact that there exist two well-defined types of new stars or novae which might be distinguished as *common novae* and *super-novae*. No intermediate objects have so far been observed.

Common novae seem to be a rather frequent phenomenon in certain stellar systems. Thus, according to Bailey,[1] ten to twenty novae flash up every year in our own Milky Way. A similar frequency (30 per year) has been found by Hubble in the well-known Andromeda nebula. A characteristic feature of these common novae is their absolute brightness (M) at maximum, which in the mean is -5.8 with a range of perhaps 3 to 4 mags. The maximum corresponds to 20,000 times the radiation of the sun. During maximum light the common novae therefore belong to the absolutely brightest stars in stellar systems. This is in full agreement with the fact that we have been able to discover this type of novae in other stellar systems near enough for us to reach stars of absolute magnitude -5 with our present optical equipment

B. Super-Novae.—The novae of the second group (super-novae) presented for a while a very curious puzzle because this type of new star was found, not only in the nearer systems, but apparently all over the accessible

range of nebular distances. Moreover, these novae presented the new feature that at their maximum brightness they emit nearly as much light as the whole nebula in which they originate. Since the investigations of Hubble and others have revealed that the absolute total luminosities of extragalactic systems scatter with rather small dispersion around the mean value $M_{vis} = -14.7$, there is no doubt that we must attribute to this group of novae an individual maximum brightness of the order of $M_{vis} = -13$.

A typical specimen of these super-novae is the well-known bright nova which appeared near the center of the Andromeda nebula in 1885 and reached a maximum apparent brightness of $m = 7.5$. Since the distance modulus of the Andromeda nebula is

$$m - M = 22.2, \qquad (1)$$

the absolute brightness of the nova at maximum was $M = -14.7$. An integration of the light-curve shows that practically the whole visible radiation is emitted during the 25 days of maximum brightness and that the total thus emitted is equivalent to 10^7 years of solar radiation of the present strength.

Finally, there exist good reasons for the assumption that at least one of the novae which have been observed in our Milky Way system belongs to the class of the super-novae. We refer to the abnormally bright nova of 1572 (Tycho Brahe's nova).[2]

About the final state of super-novae practically nothing is known. The bright nova of 1885 in the Andromeda nebula has faded away and must now be fainter than absolute magnitude -2. Repeated attempts to identify the nova of 1572 with one of the faint stars near its former position have so far not been very convincing.

Regarding the initial states of super-novae only the following meager facts are known. First, super-novae occur not only in the blurred central parts of nebulae but also in the spiral arms, which in certain cases are clearly resolved into individual stars. Secondly, the super-nova of 1572 in its initial stage probably was not brighter than apparent magnitude 5 as otherwise it would be registered as such in the old catalogues, which, however, is not the case.

Super-novae are a much less frequent phenomenon than common novae. So far as the present observational evidence goes, their frequency is of the order of one super-nova per stellar system (nebula) per several centuries.

We believe that on the basis of the available observations of super-novae the following assumptions are admissible:

(1) Super-novae represent a general type of phenomenon, and have appeared in all stellar systems (nebulae) at all times as far back as 10^9

years. To be conservative we shall assume for purposes of calculation that in every stellar system only one super-nova appears per thousand years.

(2) Super-novae, initially, are quite ordinary stars whose masses are not greater than 10^{33} gr. to 10^{35} gr.

(3) The super-nova of 1885 in Andromeda is a fair sample. We therefore base our calculations on the characteristics observed for this super-nova, namely:

(α) At maximum the visible radiation L_V emitted per second is equal to that of 6.3×10^7 suns. The radiation from our sun is

$$L_\odot = 3.78 \times 10^{33} \text{ ergs/sec.} \tag{2}$$

Therefore

$$L_V = 6.3 \times 10^7 L_\odot = 2.38 \times 10^{41} \text{ ergs/sec.} \tag{3}$$

The *total* visible radiation which was emitted by our super-nova represents an energy $E_V = 10^7$ years of $L_\odot$, that is

$$E_V = 1.19 \times 10^{48} \text{ ergs.} \tag{4}$$

(β) A common nova reaches maximum brightness in about two to three days. Indications are that a super-nova reaches maximum brightness during about the same interval.

C. Total Radiation from a Super-Nova.—In order to obtain an estimate of the total radiation, visible and invisible, from a super-nova, we consider two idealized limiting cases.

First Case.—We assume that the observed L_V corresponds to the integrated red end of a black body radiation of the effective temperature T_e, which we proceed to determine. We shall later make use of the further assumption that the total energy emitted from the super-nova is at least as great as that of the black body radiation at the temperature T_e, the red end of which is equal to that of the observed visible radiation from the super-nova. The integration over the red tail will be taken from the frequency $\nu = 0$ to the violet end ν_v of the visible spectrum; that is, to $\nu_v = 7.5 \times 10^{14}$ sec.$^{-1}$. The temperature resulting from our calculations justifies the introduction of the Rayleigh-Jeans law, namely,

$$I_\nu = \frac{2\pi k T_e \nu^2}{c^2}, \tag{5}$$

into the integration mentioned, so that

$$L_V = S \int_0^{\nu_v} I_\nu d\nu = 1.34 \times 10^8 \, S T_e, \tag{6}$$

where S is the surface of the super-nova. If the initial stage (i) of the super-nova is a star similar to our sun, its radius would be

$$R_i = 6.95 \times 10^{10} \text{ cm.} = R_\odot. \tag{7}$$

At maximum brightness our super-novae will be considerably blown up and its radius R must then be considerably greater than $R_\odot$. Reasons can be advanced, however, for supposing that probably

$$R < 100\ R_\odot = R_1, \tag{8}$$

and that almost certainly

$$R < 400\ R_\odot = R_2. \tag{9}$$

This estimate of the radius R of a super-nova at maximum brightness is based on a comparison with common novae.[3] Roughly speaking, a common nova radiates like a black body until a radius R_m is reached such that

$$R_1 < R_m < R_2. \tag{10}$$

For $R = R_m$ the brightness of the common nova is at its maximum. For $R > R_m$ extremely wide (50 to 100 Å) emission lines appear and gaseous shells are expelled from the nova at great speeds. Also the brightness of the nova declines; on the other hand the observed ionization of the gaseous shells indicates that a vast amount of ultra-violet radiation is emitted at this stage of the expansion. Furthermore, there are indications that R_m is the smaller the greater the speed with which the nova initially blows up. As a super-nova blows up faster than a common nova, we feel safe in assuming that R_m for a super-nova cannot be greater, and probably is smaller, than R_m for a common nova. To be conservative, we shall carry out our calculations for the two cases

$$R_m = R_1 = 7 \times 10^{12} \text{ cm. and } R_m = R_2 = 2.8 \times 10^{13} \text{ cm.} \tag{11}$$

The corresponding surfaces are

$$S_1 = 6.15 \times 10^{26} \text{ cm.}^2 \text{ and } S_2 = 9.84 \times 10^{27} \text{ cm.}^2 \tag{12}$$

With these figures we obtain from (6), (8) and (9) the effective surface temperature

$$\left.\begin{aligned} &T_e = L_V/1.34 \times 10^8 S = 1.78 \times 10^{33}/S, \\ &T_1 = 2.89 \times 10^5 \text{ degrees}, \quad T_2 = 1.81 \times 10^5 \text{ degrees.} \end{aligned}\right\} \tag{13}$$

From T we obtain the total radiation per second from our sample super-nova:

$$\left.\begin{aligned} &L_T = S\frac{ac}{4}T_e^4 = 1.80 \times 10^{-37} L_V^4 S^{-3} = 5.75 \times 10^{128} S^{-3} \\ &L_{T_1} = 2.46 \times 10^{48} \text{ ergs/sec.}, \quad L_{T_2} = 5.98 \times 10^{44} \text{ ergs/sec.}, \end{aligned}\right\} \tag{14}$$

where $a = 7.63 \times 10^{-15}$ ergs is the Stefan-Boltzmann radiation constant,

and c the velocity of light. The total energy emitted during the existence of the super-nova therefore is of the order of

$$\left.\begin{aligned} &E_T = L_T\, E_V/L_V = 5 \times 10^6\, L_T \\ &E_{T_1} = 12.3 \times 10^{54}\,\text{ergs}, \quad E_{T_2} = 2.99 \times 10^{51}\,\text{ergs}. \end{aligned}\right\} \quad (15)$$

These values correspond to a loss of mass

$$\Delta M = E_T/c^2, \quad \Delta M_1 = 1.37 \times 10^{34}\,\text{gr.}, \quad \Delta M_2 = 3.32 \times 10^{30}\,\text{gr.} \quad (16)$$

In reality the mass radiated away may be several times this amount, and it therefore becomes evident that *the phenomenon of a super-nova represents the transition of an ordinary star into a body of considerably smaller mass.*

Second Case.—It might be objected that we have far over-estimated the effective temperature T_e of the super-nova, inasmuch as the surface, for some reason or other, may be so large that approximately $L_T = L_V$, in which case T_e would be approximately equal to the effective temperature of the sun. As the maximum of L_V corresponds to about 7×10^7 times the radiation from the sun, the radius R of the super-nova at maximum brightness would be

$$R = 8.36 \times 10^3\, R_\odot = 5.81 \times 10^{14}\,\text{cm}. \quad (17)$$

If the super-nova is initially an ordinary star which in about one day blows up to the radius R, the velocity of expansion of the surface will be of the order of

$$v = 6.72 \times 10^9\,\text{cm./sec.}, \quad (18)$$

which, per *proton*, gives the kinetic energy

$$E_K = m_p\, v^2/2 = 0.05\, m_p c^2. \quad (19)$$

It appears, therefore, that the kinetic energies E_K of the individual particles would be quite comparable with the energy of annihilation mc^2 of these particles.

Furthermore, in order to produce such energies, the radiation trapped inside the opaque surface must correspond to average temperatures T_i which are considerably higher than T_e. From this it would follow that the energy of the trapped radiation $\left(\frac{4\pi}{3} R^3 a T_i^4\right)$ alone is comparable with energy of total annihilation of the star (10^{54} ergs).

The above considerations seem to indicate that in any case the total energy emitted in the super-nova process represents a considerable fraction of the star's mass. We also think that our case (1) corresponds more nearly to the reality than does case (2). A more detailed discussion of the super-nova process must be postponed until accurate light-curves and high-dispersion spectra are available.

Unfortunately, at the present time only a few underexposed spectra of super-novae are available, and it has not thus far been possible to interpret them.

[1] S. I. Bailey, *Pop. Astr.*, **29**, 554 (1921).
[2] K. Lundmark, *Kungl. Svenska Vetensk. Handlingar*, **60**, No. 8 (1919).
[3] *Handbuch d. Astrophysik*, Vol. VI (Novae).

COSMIC RAYS FROM SUPER-NOVAE

BY W. BAADE AND F. ZWICKY

MOUNT WILSON OBSERVATORY, CARNEGIE INSTITUTION OF WASHINGTON AND CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA

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A. Introduction.—Two important facts support the view that cosmic rays are of extragalactic origin, if, for the moment, we disregard the possibility that the earth may possess a very high and self-renewing electrostatic potential with respect to interstellar space.

(1) The intensity of cosmic rays is practically independent of time. This fact indicates that the origin of these rays can be sought neither in the sun nor in any of the objects of our own Milky Way.

(2) The decrease in intensity of cosmic rays in equatorial regions has successfully been explained by assuming that at least a part of the rays consists of very energetic, positively or negatively charged particles. These particles must be of extra-terrestrial origin, as otherwise the distance traversed by them would not be long enough for the earth's magnetic field to produce the observed dip in intensity at the equator.

From the fact that in the cloud-chamber experiments no protons or charged particles heavier than electrons have been observed in any considerable number, one might conclude that the corpuscular component of cosmic rays consists of positive or negative electrons, or both. The characteristics of the east-west effect indicate that the positively charged particles far outnumber the negatives. However, whether or not these particles are electrons cannot as yet be said with certainty, since the electrons which are observed in cloud chambers may all be secondary particles formed in the earth's atmosphere by different primaries.

With the facts mentioned as a beginning it has become customary to reason approximately as follows. Since none of the objects of our Milky Way seem to produce any cosmic rays, these rays probably are not emitted from any of the extragalactic nebulae either, as the spirals among these

nebulae are in most respects similar to our own Milky Way. One arrives therefore at one of two hypotheses, either that cosmic rays originate in intergalactic space or that they are survivors from a time when physical conditions in the universe were entirely different from what they are now (Lemaître). On closer scrutiny both hypotheses prove to be very unsatisfactory. On both views one is forced to assume entirely fantastic processes as regards the mode of creation of the rays. Furthermore, on neither of the two hypotheses can it easily be understood why the ratio of the intensity of cosmic rays to the intensity of visible light from extragalactic space is so much greater than unity, whereas the same ratio for our own galaxy is certainly smaller than one (probably less than 0.005).[1]

In the following we make an entirely new proposal, which, we think, removes some of the major difficulties concerning the origin of cosmic rays.

It has been concluded that extragalactic nebulae cannot be the centers of production of cosmic rays, as no such rays seem to originate in our own galaxy. If, however, the production of cosmic rays is related to some sporadic process, such as the flare-up of a super-nova, the above-mentioned difficulties disappear. We shall try to show that this hypothesis enables us to derive the intensity of the cosmic rays which arrive on the earth, and that direct observations of intensity are in fair agreement with the value thus computed.

B. Intensity of Cosmic Rays.—The considerations of the preceding paper (these PROCEEDINGS, **20**, 254 (1934)) suggest that in order to produce the stupendous radiation of a super-nova each particle of mass m must on the average contribute energy of the order

$$\overline{U} = 0.1\, mc^2, \qquad (1)$$

which per proton corresponds to an energy of approximately 10^8 volts. Individual photons or material particles ejected from the super-nova may of course possess energies much greater than $\overline{U}$. We therefore feel justified in advancing tentatively the hypothesis that *cosmic rays are produced in the super-nova process*. It also seems reasonable to assume that a considerable part of the total radiation E_T is emitted in the form of very hard rays or energetic particles. During the passage through the extremely tenuous material parts of the super-nova some of the cosmic rays will be stopped or softened, thus heating up the material of the super-nova to the temperature T. The escape of the cosmic rays must be pictured as a large-scale analogue to the escape of the various hard rays from a radioactive substance with a resulting heating of the substance.

For the purpose of further calculations we assume that cosmic rays are created *only* in super-novae, with the following consequences.

First, it can now be understood why our own galaxy has emitted no cosmic rays during the years in which these rays have been observed.

The reason is simply that no super-nova eruption occurred in our galaxy during this period.

Second, we can estimate the intensity of the cosmic rays reaching the earth, assuming that every super-nova emits in the form of cosmic rays a total amount of energy of the order of E_T. See equation (15) of the preceding paper.

It has previously been shown[1] that, if radiation is emitted uniformly throughout the universe at the constant rate of E ergs/cm.^{3} sec., the intensity of this radiation reaching the earth is

$$\sigma = E\,D/8, \tag{2}$$

where, approximately, $D = 2 \times 10^9$ L.Y. (light years). This formula was derived on the assumption of a red-shift which is proportional to the distance of the source of emission. If the red-shift is caused by an actual expansion of the universe, certain corrections must be applied to the expression (2). These corrections, which do not materially alter our conclusions, may be found in a paper by Professor P. S. Epstein (these PROCEEDINGS, **20**, 67 (1934)).

Since the distribution of nebulae in space corresponds approximately to one nebula in a cube whose edge is $l = 10^6$ L.Y., we obtain

$$\sigma = E_T\,D/8\,l^3\tau, \tag{3}$$

where, in accordance with the observed frequency of occurrence of super-novae in a given nebula, we must put $\tau = 1000$ years. If we insert $E_T = 10^{53}$ to 10^{54} ergs, which according to the preceding paper are probable values of E_T, we obtain

$$\sigma = 0.8 \times 10^{-3} \text{ to } 8 \times 10^{-3} \text{ ergs/cm.}^2 \text{ sec.} \tag{4}$$

The observations of the intensity of cosmic rays made by E. Regener[2] (based on ion counts) give

$$\sigma' = 3.53 \times 10^{-3} \text{ ergs/cm.}^2 \text{ sec.}, \tag{5}$$

whereas Millikan, Bowen and Neher's value[3] is

$$\sigma'' = 3.2 \times 10^{-3} \text{ ergs/cm.}^2 \text{ sec.}, \tag{6}$$

Our hypothesis is therefore in surprisingly good agreement with the direct observations of the intensity of cosmic rays.

Although we ourselves are by no means convinced that the universe is expanding, it must be stated that the above results are not in contradiction with the short time scale of the order of 10^9 years demanded by some of the relativistic cosmologies.

If the initial "spectral" distribution of the cosmic rays leaving a super-nova could be determined, the spectral distribution of the rays reaching

the earth could be obtained by taking into account the effect of the red-shift.

If a super-nova should again occur in our Milky Way system, the intensity of the cosmic rays would be considerably altered for the period of a few days. The change in intensity $\Delta\sigma$ would be

$$\Delta\sigma = L_T/4\pi \times 10^{42} r^2 \text{ ergs/cm.}^2 \text{ sec.}, \quad (7)$$

where r is measured in units of 1000 L.Y. Numerically the change is of the order of

$$\Delta\sigma = 10^4/r^2 \text{ ergs/cm.}^2 \text{ sec.}, \quad (8)$$

since, according to the preceding paper, 10^{47} ergs/sec. seems a probable value of L_T. Supposing that the super-nova occurs in the neighborhood of the center of our own galactic system, that is, $r = 30$, approximately, we obtain

$$\Delta\sigma = 11 \text{ ergs/cm.}^2 \text{ sec.} \sim 10^4\,\sigma. \quad (9)$$

If interest in these questions still prevails at that future time, science will therefore be able to test the correctness of our hypothesis some time during the next thousand years or so, as the occurrence of a super-nova in our own system would multiply the intensity of the cosmic rays by a factor one thousand or more. It also seems quite possible to observe with cosmic-ray electroscopes the flare-up of a super-nova in one of the nearer extragalactic nebulae, as for them $r = 1000\,n$, and

$$\Delta\sigma = 0.01/n^2 \text{ ergs/cm.}^2 \text{ sec.}, \quad (10)$$

where n is a number of the order one. It might in this connection be of interest to follow up the causes for Regener's[4] curious balloon observation of March 29, 1933.

Furthermore, we recommend that observers of cosmic rays be on the lookout for short-period systematic increases in the intensity of cosmic rays in order to determine as accurately as possible the time and the direction of the maximum intensity. With such data quickly at hand, astronomers might be able actually to locate the responsible super-nova in one of our near-by systems. As there are about one thousand nebulae in the region

$$0 < n < 10, \quad (11)$$

one super-nova per year should be expected in this "immediate" neighborhood of ours, producing an intensity increase in the cosmic rays of the order of one per cent or more for a period of a few days.

C. Additional Remarks.—A more detailed critical discussion of the views

advanced in this article must be postponed because of lack of space. We wish to say only

(1) So far we cannot offer any satisfactory explanation of the east-west effect.

(2) It remains to be explained why the dust and gas clouds which lie along the principal plane of our own galaxy do not appreciably absorb the cosmic rays.[5] This point, however, needs further observational testing.

In addition, the new problem of developing a more detailed picture of the happenings in a super-nova now confronts us. With all reserve we advance the view that a super-nova represents the transition of an ordinary star into a *neutron star*, consisting mainly of neutrons. Such a star may possess a very small radius and an extremely high density. As neutrons can be packed much more closely than ordinary nuclei and electrons, the "gravitational packing" energy in a *cold* neutron star may become very large, and, under certain circumstances, may far exceed the ordinary nuclear packing fractions. A neutron star would therefore represent the most stable configuration of matter as such. The consequences of this hypothesis will be developed in another place, where also will be mentioned some observations that tend to support the idea of stellar bodies made up mainly of neutrons.

D. Conclusions.—From the data available on super-novae we conclude

(1) Mass may be *annihilated* in bulk. By this we mean that an assembly of atoms whose total mass is M may lose in the form of electromagnetic radiation and kinetic energy an amount of energy E_T which probably cannot be accounted for by the liberation of known nuclear packing fractions. Several interpretations of this result are possible and will be published in another place.

(2) The hypothesis that *super-novae emit cosmic rays* leads to a very satisfactory agreement with some of the major observations on cosmic rays.

Our two conclusions are essentially independent of each other and should perhaps be judged separately, each on its respective merits.

[1] F. Zwicky, *Phys. Rev.*, **43**, 147 (1933).
[2] E. Regener, *Zeit. f. Phys.*, **80**, 666 (1933).
[3] R. A. Millikan, I. S. Bowen and H. V. Neher, *Phys. Rev.*, **44**, 246 (1933).
[4] E. Regener, *Nature*, **132**, 696 (1933).
[5] F. Zwicky, *Helvetica Physica Acta*, **6**, 110 (1933).

THE VELOCITY-DISTANCE RELATION FOR ISOLATED EXTRAGALACTIC NEBULAE

BY EDWIN HUBBLE AND MILTON L. HUMASON

MOUNT WILSON OBSERVATORY, CARNEGIE INSTITUTION OF WASHINGTON

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The velocity-distance relation was first established[1] for a few nebulae whose distances were derived from involved stars. With the aid of data from clusters whose relative distances were estimated from the mean apparent magnitudes of the many members of each cluster, the relation was confirmed in the form[2]

$$\log v = 0.2\, m + 0.51,$$

which, since

$$\log d = \frac{m - M + 5}{5},$$

leads to the linear relation

$$v = 10^{(0.2M - 0.49)} d,$$

where M is the mean absolute magnitude of cluster nebulae.

In both cases the isolated nebulae in which no stars were detected, i.e., nebulae for which total luminosity must serve as a criterion of distance, were used in general for confirming the order of the results rather than for their precise formulation. This procedure was employed because of the rather large effects resulting from the selection of isolated nebulae according to apparent magnitude, which the data were insufficient to evaluate. Mr. Humason has since accumulated velocities for 35 isolated nebulae, which, together with the 50 previously available, form a body of data adequate for the purpose.

The new data are listed in table 1, in which the magnitudes are taken from the convenient Harvard Survey of the brighter nebulae, with the exception of the 6 faintest, which represent estimates by one of the writers. The velocities, in km. per sec., are not corrected for solar motion. The data previously available may be found in the two former discussions of the velocity-distance relation.

Actually, 94 velocities are available, but 9 have been rejected, of which 7 belong to our own local group and give velocities that are mere reflections of the rotation of the galactic system (the two satellites of the galactic system, namely, the Magellanic Clouds, M 31 with its two satellites, M 33, and NGC 6822); one, IC 342, a large open spiral at latitude $+12°$ and obviously affected by local obscuration, may belong to the same category;

and finally NGC 404, $v = +40$ (corrected for solar motion), $m_{pg} = 12.0$, an exceptional case which would unduly influence the correlation under investigation. NGC 404 is not necessarily inconsistent, since permissible values of absolute magnitude and peculiar motion may be assigned which would account for the apparent discrepancy.

TABLE 1

RADIAL VELOCITIES OF ISOLATED NEBULAE

NGC	TYPE	m_{pg}	v	NGC	TYPE	m_{pg}	v
157	Sc	11.2	+1800	3377	E5	11.6	+ 650
628	Sc	11.2	600	3384	SBa	11.3	850
720	E5	11.7	1800	3412	SBa	11.6	950
722	Sb	12.0	2200	3414	SBb	12.2	1450
1084	Sc	11.2	1450	3486	Sc	11.4	1250
1087	Sc	11.2	1850	N_2	E	17.5*	19000
1332	Sa	11.4	1400	3726	Sc	11.7	1150
1400	E1	12.5	500	5253	Ir**	10.8	400
1407	E0	11.5	+2000	5846	E0	11.6	1700
IC 342	Sc	(14.0)*	− 25	5982	E3	12.7*	+2900
N_1	Sa	16.0*	+4600	6946	Sc	11.1	− 150
2403	Sc	10.2	125	7177	Sbc	12.1	+1300
2655	Sa	11.6	1350	7343	SBb	14.1*	1200
2768	E6	12.0	1400	7727	Sa	12.0	1800
2775	Sa	11.5	1100	7814	Sa	12.4	+1000
2787	SBa	12.1	700				
2903	Sc	10.3	350				
3147	Sc	11.9	2600				
3344	SBc	11.9	550				
3351	SBb	11.5	+ 700				

* Magnitudes estimated at Mount Wilson; that for IC 342 is very uncertain.

** Emission spectrum. Type uncertain since the possibility that the object is a planetary is not ruled out. The point is important because of the nova Z Centauri, which appeared in this nebula and attained a maximum many times the total luminosity of the nebula.

N_1 and N_2 are faint nebulae at R. A. = $4^h\ 37^m0$; Dec. = $+4°\ 7'$ and R. A. = $10^h\ 54^m0$; Dec. = $+57°\ 12'$ (1930), respectively.

N_2 is within the field of a very faint cluster found by Dr. Baade but is about 0.5 mag. brighter than the estimated upper limit of the cluster nebulae. The spectrum was observed on the slight chance that the nebula might be an outstanding member of the cluster. The red-shift actually observed is about half the value predicted for the cluster and hence the nebula is very probably a normal field nebula.

Figure 1 exhibits the correlation between m (from the Harvard Survey or from Mount Wilson estimates), corrected for local obscuration,[3] and $\log v$, corrected for solar motion,[1] for the 85 remaining nebulae. A linear

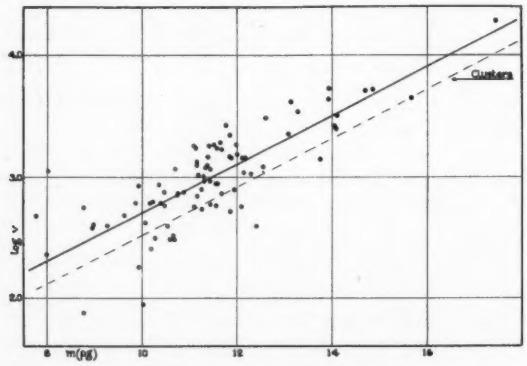
correlation is evident, and a least-squares solution, with the data grouped according to log v, leads to the relation

$$\log v = (0.203 \pm 0.010)m + 0.67.$$

When the precisely linear coefficient of m, namely, 0.2, is adopted, the relation, together with that derived from the clusters, is

Isolated Neb.	$\log v = 0.2m + 0.71$
Clusters	$\log v = 0.2m + 0.51$
	0.20
$\sigma = 0.862$	$0.2 \times 1.38\sigma^2 = 0.20$

The significance of σ, the dispersion, will be discussed later. The relation for isolated nebulae parallels that for the clusters but is displaced just one



Velocity-Distance Relation for Isolated Nebulae

FIGURE 1

magnitude toward the brighter side. This displacement represents the effect of selection.

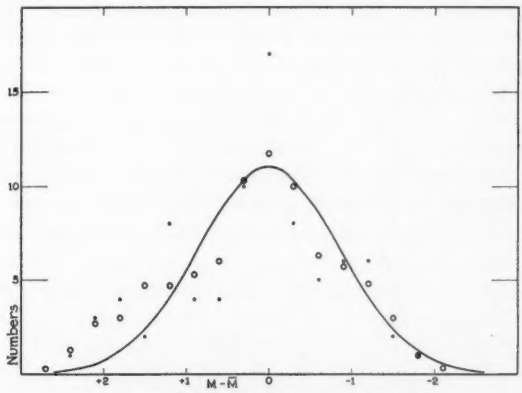
The luminosity function of nebulae (the frequency distribution of absolute magnitudes for the nebulae in a given volume of space) approximates a normal error-curve with a maximum at M_0 and with a dispersion rather uncertainly estimated at less than a magnitude. The density function, at least on the grand scale, is approximately constant. These conditions represent the classical example of the simplest case in statistical studies of stellar distribution, which has been fully discussed by Malmquist.[4] The point of immediate interest is the frequency distribution of absolute magnitudes among nebulae of a given apparent magnitude. This function is found to be another normal error-curve, with the same dispersion as the

luminosity function but with the maximum, $\overline{M}$, displaced toward the brighter side by the amount

$$M_0 - \overline{M} = 1.38\sigma^2,$$

where the coefficient is $0.6 \log_e 10$. In other words, when nebulae are selected on the basis of apparent magnitude, the absolute magnitudes are too bright by the systematic term $1.38\sigma^2$.

Since the effect of selection applies to the isolated nebulae and not to the



Frequency Distribution of Residuals in Velocity-Distance Relation

FIGURE 2

The smooth curve is a normal error-curve with a dispersion of 0.86. Numbers of nebulae in intervals of 0.3 mag. are represented by dots; overlapping means for three successive intervals, by open circles. Two nebulae fall outside the limits of the diagram: On the brighter side, NGC 4594, whose magnitude is very uncertain; on the fainter side, NGC 6946, whose observed velocity is small compared with the average peculiar motion of nebulae.

clusters, the observed displacement in the velocity-distance relation is in the direction and of the order to be expected. The displacement represents a dispersion

$$\sigma = \sqrt{\frac{0.20}{0.2 \times 1.38}} = 0.86,$$

which agrees with the dispersion derived from the residuals themselves, namely, 0.862. The frequency distribution of the residuals is exhibited in figure 2. The precise agreement is a coincidence, since the residuals include all errors and peculiar motions and hence lead to a value larger than the true dispersion, while the data for the clusters when slightly revised

for galactic obscuration, etc., give a slightly smaller displacement. These second-order corrections, together with the effect of magnitude errors, will be more fully discussed in a *Contribution* from the Mount Wilson Observatory.

Meanwhile it is evident that, within the small uncertainties, the isolated nebulae exhibit the same relation as the cluster nebulae and hence that their luminosity functions are closely similar. The important quantity M_0 is most reliably derived from the Virgo Cluster whose distance is indicated by stars involved in the later-type spirals. Reëxamination of data available at present suggests no significant revision of the earlier estimate,[2] -13.8, although the round number -14 might be used with about equal justification pending the detailed analysis of the Virgo Cluster.

The statistical correction, $M_0 - \overline{M}$, affects investigations of distribution by counts of nebulae and reduces the previously[3] estimated values of the density of matter in the observable region by a factor of the order of three. At great distances the correction diminishes as the red-shifts increase, and beyond about the nineteenth magnitude the variation cannot be ignored.

[1] *Mount Wilson Comm.*, No. 105; *Proc. Nat. Acad. Sci.*, **15**, 168–173 (1929).

[2] *Mount Wilson Contr.*, No. 427; *Astrophys. J.*, **74**, 43–80 (1931).

[3] Magnitudes are corrected for galactic latitude by the cosecant law $\Delta m = 0.15$ (cosec $\beta - 1$). *Mt. Wilson Contr.*, No. 485; *Astrophys. J.*, **79**, 8–76 (1934). The corrections are required by the fact that most of the faint nebulae are in very low latitudes.

[4] K. G. Malmquist, "On Some Relations in Stellar Statistics," *Arkiv för Matematik. Astronomi och Fysik*, Stockholm, **16**, No. 23 (1921).

HEAT INDUCED MUTATIONS IN DROSOPHILA

By H. H. Plough and P. T. Ives

Department of Biology, Amherst College

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Although irradiation by x-rays remains the only reliable method for inducing mutations artificially, the discovery by Goldschmidt[1] that gene mutations were induced by exposing larvae of *Drosophila* for 24 hours to a sub-lethal temperature of 36° has stimulated interest in the problem of the origin of mutations in nature. The result had been suggested by the previous work of Muller and Altenberg[2] and of Muller,[3] but the results of Goldschmidt and more recently of his co-worker Jollos[4] constitute an important contribution to genetics which is the more interesting since there have been so many previous breeding experiments with *Drosophila* at

high temperatures. Since Muller and Mott-Smith[5] conclude that natural radiation is inadequate to account for mutations in nature, it seems possible to suggest that ubiquitous temperature variations may play that rôle.

Jollos has carried the work much further, and he believes that his data show the induction of specific and corresponding cytoplasmic and gene changes, as well as the calling forth of directed (gerichtete) mutations and their enhancement in a definite direction. His views are paraphrased by Goldschmidt ([6], p. 547): "The genes produce within the protoplasm active stuffs which are of the same constitution as the genes themselves. Both will react in the same way upon external conditions, but those within the protoplasm easier than those protected within the chromosomes."* Finally, he believes, successive allelomorphs of the same gene are produced by repeated exposures to high temperature which result in more and more extreme manifestations of the same characters. Jollos concludes not only that exposure to high temperature causes mutations, but that his results furnish the experimental basis for orthogenetic or straight line series in evolution in cases where the characters are favored by natural selection.

Such ambitious claims have naturally aroused much interest and a good deal of skepticism. Obviously it is desirable that the work should be repeated and the conclusions independently tested. This has been done by a number of Drosophilists with somewhat conflicting results. (Cf. Muller[7] for references.) Our own tests of the Goldschmidt method of treatment were begun by the senior author in 1930 and the junior author has borne the greater share of the work during the past two years. So far as possible we have followed the method of Goldschmidt and Jollos exactly, but we have kept somewhat more extensive records, at least than the latter has so far published. Certain of our results have been reported at intervals in abstract form to meetings of the Genetics Society,[8] and the full report will appear in *Genetics*. The publication date is, however, so far in the future that it seems desirable to make available a summary at this time.

In all, 10 separate experiments were run in which one or both parents were treated with high temperatures following the Goldschmidt method. These involved several wild stocks including the Florida culture, mutant stocks having gene mutations from Chromosomes I, II and III, and the X lethal testing stocks XX yellow and Cl B. In several experiments crossing-over data were assembled in addition to data on lethal recessive and dominant mutations. In several of the experiments the series were continued for as many as 10 generations after treatment. In every test all somatic variations of any sort were recorded. All were mated and their offspring followed for at least two generations. When a mutation occurred it was located by the usual methods, and if it appeared to be an allelomorph of a known gene, its allelomorphism was established by crosses to the proper stock when that was available. Controls were run for all the series, and

they were continued with the same tests of variations for the same number of generations as the similar heated stocks. We thus have a considerable body of data, from which are available comparisons of mutation rates for all types of mutations, and also comparisons of the relative numbers and kinds of somatic or non-inherited variations. Altogether the totals of all these experiments are in excess of 200,000 flies in heated lines and close to 100,000 control flies. These constitute a mass of data bearing on the effect of brief exposures to sub-lethal temperature on mutation which seems adequate for conclusive answers to the questions raised.

TABLE 1

	NO. MUTATIONS FOUND					
TOTAL NO. FLIES	(A) X LINKED LETHALS	(B) X LINKED RECESSIVES	(C) AUTOSOMAL RECESSIVES	(D) VISIBLE DOMINANTS	(E) CHROMOSOMAL ABERRATIONS	TOTAL NO. MUTATIONS / TOTAL NO. FLIES
186,426	13	7	9	13	2	$\frac{44}{186,426} = 0.000236 \pm 0.0000240$
73,221	1	1	0	1	0	$\frac{3}{73,221} = 0.000195 \pm 0.0000283$

In table 1 are summarized the mutations found in the first five generations of the lines heated once and the corresponding controls. The difference is here 6.7 times the probable error of the difference, which makes the chance that the two values are identical of the order of 1:250,000. Since the data indicate that the number of mutations in the heated lines is nearly six times greater than in the controls, this constitutes clear confirmation of the Goldschmidt-Jollos results. Taking into account the number of tests of each of the various kinds of mutations, we find that the visible dominants (d) show the lowest frequency of mutation and that autosomal recessives (c) and X linked lethals (a) show a frequency about 150 times greater. In these totals no mutations are included which appeared in both heated and control lines, nor are doubtful or irregular cases. The totals therefore show minimal rather than maximal values.

In all, visible mutations were found in 24 loci in all chromosomes, and X linked lethals in 10 loci. Most of these loci were known, but in several cases new allelomorphs were found. The most striking of the latter, which occurred 3 times in heated lines and once in the controls, was a dominant vestigial allelomorph called vg^{No}. It has been identified by Dr. O. L. Mohr as being next in the series of vestigial allelomorphs to "no wing." In heterozygous condition it shows a notch in each wing, and in homozygous state it is wingless and the females are sterile. Another striking dominant is Glued eye (Gl), which is a new chromosome III locus close to Dichaete. At least two mutants to the dominant Star eye (Chromo-

some II) were found, while of the known recessive mutations garnet (Chromosome I) occurred in heated lines 3 times, brown (Chromosome II) twice and among others white (from normal) once. There is no evidence whatever of the appearance of allelomorphs in a step-by-step series as recorded by Jollos, even when mutated stocks were given several successive treatments. In general, the results resemble those shown in spontaneous or natural mutation except that the rate of mutation is increased about six times.

It is of interest, however, that the number of recurrent mutations found is considerably in excess of those found spontaneously. The 49 mutations which appeared in the complete series of heated lines gave a total of 34 different loci. Ten of these gave 2 or more mutations in the same locus, 3 gave 3 or more and 2 gave 4. Using the formula for calculating mutation rate (Muller[3]) these figures might be expected to fit into a simple proportion. As a matter of fact they indicate a much increased tendency for remutation in certain loci. This result indicates that the treatment with high temperature causes certain genes to mutate with a greater frequency than others. However, the mutations which have recurred most frequently in our experiments do not correspond with any of those reported as recurring by Jollos, nor indeed do his results correspond from one experiment to another. Until it can be shown that the same treatment produces consistent remutations in certain particular loci, the only safe conclusion is that exposure to high temperature increases the general mutation rate as already indicated.

Since every anomaly of any sort which occurred in the heated and control lines was tabulated and bred for two generations, accurate data are available on the number and kinds of non-genetic or somatic variations (modifications). Such variations occurred in a large percentage (often 100%) of the first generation flies directly subjected to heat. In successive generations they continue to occur in heated lines for five or six generations at a rate at least three times greater than in the control lines. Although they are in greater numbers in the heated lines, these non-inherited anomalies are not different in kind from those in the controls. In each the most common anomaly was one or another type of rough eye—the facets irregularly disarranged—and each showed a number of irregular, reduced or unsymmetrically strap-like wings. It is of interest, though probably not significant, that these correspond with the most frequently occurring mutations. While a few of these somatic variations reappeared in an occasional fly in later generations we found no single case of an "enduring modification" in the sense of Jollos. We did find clear evidence that the increased tendency to produce somatic modifications was inherited, but through the female line only. The offspring of heated males gave no increase in the number of non-genetic variations over the controls. It is of great interest

that the mutations themselves show no such relation. The number of mutations among the offspring is approximately the same whether the male or the female parent has been heated, and is approximately doubled when both parents have had the heat exposure. A portion of the data showing this contrast is summarized in table 2. These and other similar data

TABLE 2

DATA FROM EXPERIMENT X ONLY

TREATMENT	GENERATIONS NO.	NO. FLIES	NO. SOMATIC VARIATIONS	% NO. SOMATICS / NO. FLIES	DIFFERENCE (FROM CONTROL) / P.E. OF DIFFERENCE
♂ + ♀ heated	2	6,702	42	0.00627 ± 0.000650	5.0
♀ heated	2	7,407	50	0.00675 ± 0.000642	5.8
♂ heated	2 + 3	45,761	116	0.00253 ± 0.000050	−1.2
Control	2 + 3	24,197	68	0.00281 ± 0.000230	

			NO. MUTATIONS	% NO. MUTATIONS / NO. FLIES	DIFFERENCE (FROM CONTROL) / P.E. OF DIFFERENCE
♂ + ♀ heated	2 − 5	11,220	5	0.000446 ± 0.0001344	2.9
♀ heated	2 − 5	15,755	4	0.000254 ± 0.0000854	2.2
♂ heated	2 − 5	75,449	20	0.000265 ± 0.0000400	2.3
Control	2 − 5	36,024	2	0.000055 ± 0.0000265	

apparently leave no doubt that mutations result from an effect of the heat on the chromatin of either male or female germ cells, but that non-inherited somatic variations result from an effect of the heat on the cytoplasm which is carried over in the egg only and not in the sperm.

These results appear to throw doubt on the correctness of Jollos's view that external conditions like temperature react in the same way upon the same stuffs in cytoplasm and chromatin. Rather the effect seems to be non-specific, and to induce a general increase in the "natural" rate of spontaneous cytoplasmic and gene changes.

Finally a tabulation by generations of the mutations from heat treated lines indicated that 67% of them might have been produced by direct effect of the heat on the genes at the time of application. At least 33%, however, must have occurred later than the time of treatment. Since even this fraction appears to constitute a significant increase over the number of mutations in the controls, this result indicates that the heat produces changes in certain genes which only later manifest themselves as mutations. This delayed effect has not been noted following x-radiation.

While our data confirm the observations of Goldschmidt and Jollos that brief exposure to sub-lethal high temperature brings about a marked increase in mutation rate, we cannot agree that this can be expected to bring about orthogenetic evolution. As Haldane[9] has shown by mathematical analysis, mutations that are only slightly disadvantageous could

survive against selection only as a result of much higher mutations rates than are here found. On the other hand advantageous mutations would be selected without regard to this slight increase in rate.

These results suggest, however, that temperature variations in nature may be an important cause of spontaneous or natural mutations.

[1] Goldschmidt, *Biol. Zbl.*, **49**, 437–448 (1929).

[2] Muller and Altenberg, *Proc. Soc. Exp. Biol. Med.*, **17**, 10–14 (1919).

[3] Muller, *Proc. Int. Congr. Plant Sci.*, **1**, 897–921 (1928).

[4] Jollos, *Biol. Zbl.*, **50**, 542–554(1930); Suppl. Bd., *Zool. Anz.*, **5**, 252–295(1931); *Naturwis.*, **21**, 831–834(1933).

[5] Muller and Mott-Smith, *Proc. Nat. Acad. Sci.*, **16**, 277 (1930).

[6] Goldschmidt, *Science*, **78**, 539–548 (1933).

[7] Muller, *Proc. Sixth Int. Congr. Genetics*, **1**, 213–255 (1932).

[8] Plough and Ives, *Proc. Sixth Int. Congr. Genetics*, **2**, 156–158(1932); Plough, *Amer. Nat.*, **57**, abstr. (1933).

[9] Haldane, *The Causes of Evolution*, Harpers (1932).

* In a recent abstract of our work (Plough and Ives, *Amer. Nat.*, **58**) we appear to have misstated Jollos's views due to a mistranslation of his conclusions. We took issue with his supposed belief "that induced somatic variations subsequently become genetic," when in fact his view as stated several times in his papers is that of a parallel effect on genes and similar gene products in the cytoplasm. Our data agree much more closely with this interpretation as the subsequent summary shows. Our error, which we regret, was kindly called to our attention by Professor A. H. Sturtevant, and more recently by Professor Jollos himself.

BLOOD-GROUP INCOMPATIBILITY IN RABBIT EMBRYOS AND IN MAN

BY CLYDE E. KEELER AND W. E. CASTLE

HOWE LABORATORY AND BUSSEY INSTITUTION, HARVARD UNIVERSITY

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It is known that transfusion of blood from one individual to another may have disastrous results if donor and recipient belong to incompatible blood-groups. It is known, also, that blood-groups are determined by inheritance from both parents. If husband and wife happen to belong to different blood-groups, is it possible that the child may have blood incompatible with that of the mother and thus be in danger of having its blood agglutinated prior to birth? This question has often been raised but seems not to have received a conclusive answer. It is known that mothers of a particular blood-group may produce living children of any blood-group whatever, so that it seems improbable that any harmful effect follows a difference in blood-group between mother and child. The question then arises, how are harmful effects, such as may occur in

transfusion, precluded in the case of mother and child? It is true that blood, as such, does not pass directly from mother to child, or vice versa, through the placenta, but it seems possible that the agents of agglutination (agglutinogens and agglutinins) may pass through the placenta.

In a previous study[1] of the blood-groups of the rabbit, we have shown that agglutinins located in the plasma may pass through the placenta, but that agglutinogens attached to the blood-cells do not pass through the placenta. The blood-groups of the rabbit are not strictly comparable with the commonly recognized blood-groups of man, known as *O*, *A*, *B* and *AB*, but rather with the *M* and *N* immune blood-groups of man described in 1928 by Landsteiner and Levine.[2] But in both cases a similar relation seems to exist between hemagglutinogens borne in the blood-cells and hemagglutinins contained in the blood plasma, so that it is legitimate to assume that what precludes agglutination in one case would act similarly in the other.

We have previously described two agglutinogens, H_1 and H_2, found in rabbit red blood-cells. These agglutinogens are determined genetically by allelomorphs or alternative forms of the same gene, and each, when acting alone, behaves as a simple dominant unit-character. Their corresponding agglutinins, h_1 and h_2, are specific antibodies produced as an immunity response when blood-cells carrying the corresponding agglutinogens are repeatedly injected into a rabbit lacking that agglutinogen in its blood-cells. We employed these two natural agglutinogens and their corresponding immune antibodies in attacking the problem of foetal blood-group incompatibility.

The idea that anything detrimental will happen to incompatible embryos is based upon two assumptions; first, that maternal agglutinins may traverse the placenta and thus enter the blood-stream of the embryo; and second, that embryonic blood-cells are capable of agglutination.

We have previously shown, in the case of rabbits, that maternal agglutinins do pass through the placenta, and can be demonstrated in the bodies of embryos. We find that in such embryos the agglutinin received from the mother by transfer through the placenta maintains a titre equal to that of the same agglutinin in the mother's blood-serum; hence, it seems legitimate to assume that the agglutinin passes freely from the mother's blood to that of her embryos *in utero*.

We have also shown previously that embryos in as early a stage as one of 4 mm. body length do bear agglutinogens, since when the body of an embryo is pulped and treated with the appropriate agglutinin, the erythrocytes (normoblasts) agglutinate.

But not all embryos are found to contain the agglutinin which occurs in the mother's blood. If the mother's blood plasma contains agglutinin h_1, this same agglutinin will be found in all embryos *except those which*

have the H_1 agglutinogen by inheritance from their father. Or if the mother's blood contains agglutinin h_2, this will be found in all embryos *except those which have the H_2 agglutinogen* in their erythrocytes. It seems reasonable to assume that a maternal agglutinin is neutralized or destroyed in an embryo containing the antagonistic agglutinogen, but the process is so gradual that it has no harmful consequences. No blocking of the foetal circulation results. These conclusions are based on the following experiments.

Upon four occasions female rabbits of the *O* blood-group, which bore neither agglutinogen (H_1 or H_2) in their blood, received injections of blood from donors which possessed both. Consequently they developed both agglutinins (h_1 and h_2) in their blood plasma. When a good titre of these had been demonstrated in their blood, the does were mated. In two cases they were mated to purebred H_1 bucks, and in two cases they were mated to purebred H_2 bucks. During pregnancy the mothers received additional injections of H_1H_2 blood in order to maintain a high titre of each agglutinin in their blood. The one doe which was mated to an H_1 buck produced eight H_1 young, and the other doe which was mated to this same buck produced nine H_1 young. The one doe which was mated to an H_2 buck produced eight H_2 young, and the other doe, mated to this same buck, produced three H_2 young. Thus there were 28 incompatible embryos produced, each bearing an agglutinogen inherited from its father. The sera of eight H_1 young were tested and found to contain h_2 agglutinin but no h_1 agglutinin. The sera of eight H_2 young were tested and found to contain h_1 agglutinin but no h_2 agglutinin.

In a second experiment, on three occasions, does of the *O* group which had received injections of H_1H_2 blood were mated with bucks heterozygous for a single agglutinogen. In two of three matings made, the buck was heterozygous for H_1; in one mating he was heterozygous for H_2. In such matings it would be expected that half the young would inherit an agglutinogen from their father, while half would inherit no agglutinogen and would thus be *O* animals. One mating to an H_1 buck produced merely two H_1 young, while the second mating produced three H_1 young and one lacking agglutinogens. The mating to an H_2 buck produced four H_2 young and three lacking agglutinogens. Thus, thirteen young were obtained from these matings. Nine contained the agglutinogen expected to occur in half of the young, and four contained no agglutinogen. Certain of the young were tested for presence of agglutinins. These were found as expected in all cases except those in which an antagonistic agglutinogen was demonstrated. No difference was found between the young which had an agglutinogen and those which lacked it as regards size, appearance, erythrocyte count or blood picture.

We conclude that an embryo may, without harm, receive through the

placenta an agglutinin from the blood of its mother antagonistic to an agglutinogen which the embryo itself possesses through inheritance from the father, but that such agglutinin will be continuously neutralized in the circulation of the foetus so that at no time does it attain titre there sufficient to agglutinate the blood-cells of the embryo.

In the common blood-groups of man agglutinins are natural, not induced, constituents of the blood plasma, but it is well known that an agglutinin is invariably wanting when the corresponding (antagonistic) agglutinogen is present. It seems reasonable to assume that here, as in the rabbit, the agglutinin, if produced at all in the presence of the agglutinogen, is continuously neutralized, and that when such agglutinin passes from the mother's blood into that of an "incompatible" embryo, it is continuously neutralized there also by the antagonistic agglutinogen.

[1] *Proc. Nat. Acad. Sci.*, **19**, 92–100, 403–411 (1933).

[2] *Jour. Exp. Med.*, **48**, 731–749 (1928).

LINKAGE STUDIES OF BRACHYURY (SHORT TAIL) IN THE HOUSE MOUSE

By Frank H. Clark

Bussey Institution, Harvard University

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A gene mutation of the house mouse known as brachyury (short tail) has been described by Dobrovolskaia-Zavadskaia[1] and has been studied extensively by her in collaboration with Kobozieff.[2] The mutation made its appearance among the descendants of individuals subjected to x-ray treatment, and may or may not have owed its production to that agency. It behaves as a dominant character in heredity and is lethal when homozygous. It finds visible expression in a variety of forms called anoury, filiform tail, kinky or bent tail, helicoid, etc. These various phenotypes are thought to be due to a single gene, the manifestations of which are influenced by modifying genes. Mice carrying this gene have been generously supplied by its discoverer to several American laboratories. Chesley,[3] at Columbia University, has studied the early embryology of brachyuric mice, finding that homozygous individuals have an abnormal development of the neural tube involving bleb formation and absent somites, and that they die about the tenth day.

My own observations on brachyuric mice have been directed toward a study of possible linkage relations of the brachyury gene with other known genes of the house mouse. Although the results of such studies are thus

far negative, it seems important that they be put on record as showing in what chromosomes of the mouse identifiable by known genes the brachyury gene does *not* lie. It may thus become the marker of another mouse chromosome. Twelve, or possibly fourteen, of the twenty mouse chromosomes are at present marked in this way. It will be shown that the brachyury gene segregates freely with reference to all of these, and accordingly must lie in a different (thirteenth or fifteenth) chromosome.

When a brachyuric mouse (invariably a heterozygote, since homozygous brachyuric embryos perish before birth) is mated with a normal individual, approximately half of the young are affected with brachyury, though they vary greatly in the length and the shape of the tail. This shows that heterozygous brachyurics have good viability. In a total of 1238 young which had one parent brachyuric, 620 were brachyuric, 618 normal. This is a perfect 1:1 ratio.

TABLE 1

BACK-CROSS DATA FROM TESTS FOR LINKAGE BETWEEN BRACHYURY AND TWELVE OTHER MUTANT CHARACTERS. X INDICATES THE DOMINANT ALLELOMORPH OF THE CHARACTER BEING TESTED; x ITS RECESSIVE ALLELOMORPH. NUMERALS IN ITALICS INDICATE CROSSOVER CLASSES

	BRACHYURIC X	BRACHYURIC x	NORMAL X	NORMAL x	PER CENT CROSSOVERS	DEV./P.E.
Flexed-tail (anemia)	66	*53*	*48*	49	46.8	1.4
Albinism	*18*	22	18	*19*	48.1	0.5
Chocolate	26	*27*	*26*	28	49.5	0.1
Leaden	17	*26*	*27*	35	50.5	0.1
Dilution	32	*32*	*28*	30	49.2	0.3
Roberts' pink-eye	26	*20*	*26*	26	47.0	0.9
Naked	*21*	20	28	*29*	51.0	0.3
Rodless	28	*21*	*24*	26	45.5	1.3
Waltzing	27	*24*	*16*	19	46.5	1.0
Zavadskaia shaker	23	*22*	*25*	18	53.4	1.0
Hairless	19	*16*	*15*	21	43.7	1.1
Dominant spotting	*11*	23	13	*24*	49.3	0.1

Tests for linkage between brachyury and twelve other mutant characters are summarized in table 1. In each experiment a cross was first made which would bring brachyury and the other mutant character into the same zygote, each in heterozygous state. The double heterozygote was then crossed with a double recessive individual, the expected result being the production of four phenotypes, two of which would be *non-crossovers* (showing the two mutant characters in the *same* relationship as before the cross was made), two other classes being crossovers (showing the characters in *changed* relationships). In table 1 the crossover classes are indicated by italic numerals, non-crossovers by Arabic numerals; also the *dominant* allelomorph of the character under test for linkage is indicated by X, its *recessive* allelomorph by x.

The back-cross populations produced in these twelve experiments vary in size from 71 in the cross with hairless to 216 in the cross with flexed tail anemia. There is an excess of crossovers in three experiments, those involving leaden, naked and Zavadskaia shaker, a deviation from equality opposite in character to that to be expected if linkage were involved. In seven other backcrosses there is a deficiency of crossovers no greater than the excess of crossovers in the cases already mentioned, and so likewise to be ascribed to chance rather than to linkage. These are the crosses involving respectively anemia, albinism, chocolate, dilution, Roberts' pink-eye, waltzing and dominant spotting. Finally in two backcrosses the deficiency of crossovers is somewhat greater, there being 45.5 per cent of crossovers in the case of rodless, and 43.7 per cent in the case of hairless. But in neither of these cases is the deviation from expectation significantly large. It is 1.3 times the probable error in the case of rodless, and only 1.1 times the probable error in the case of hairless.

These results afford satisfactory evidence that brachyury is not linked genetically with any of the twelve mutant characters mentioned in table 1. If it is not linked with any of these, it should not be linked with any mutant characters known to be linked with one or another of the twelve. For instance, albinism is known to be linked with pink-eye and shaker. Brachyury having shown no indications of linkage with albinism, it has been considered unnecessary to test for linkage with pink-eye or shaker. Roberts' pink-eye (according to unpublished observations of Roberts) is linked with agouti, which therefore has not been included in the linkage tests, since Roberts' pink-eye shows no linkage with brachyury. Piebald is linked with hairless, and short ear with dilution. Piebald and short ear have accordingly been omitted from the linkage tests.

In addition to the tests summarized in table 1, tests have also been made for possible linkage with dwarf and hydrocephalus. These present special difficulties because both mutants are infertile when homozygous, and are invisible when present as heterozygotes, since they are recessive in character. Accordingly back-cross matings to the double recessive genotype are impossible, and linkage data can be obtained only from F_2 populations.

A cross is first made between a brachyuric individual and one heterozygous for dwarf or hydrocephalus, respectively. The F_1 brachyuric individuals are then tested individually for presence of the other mutant character (dwarf or hydrocephalus). Such individuals as carry it are double heterozygotes and bear the mutant characters in the repulsion relationship. They are now mated with non-brachyuric F_1 individuals, half of which carry the other mutant character (dwarf or hydrocephalus, as the case may be). Matings which produce dwarf (or hydrocephalus) offspring give the required data on linkage.

From a cross with dwarf the F_2 numbers are shown in table 2. In no

TABLE 2

TESTS FOR LINKAGE BETWEEN BRACHYURY AND DWARF

	BRACHYURIC NORMAL	BRACHYURIC DWARF	NORMAL NORMAL	NORMAL DWARF
Observed	15	3	23	7
Expected	18	6	18	6
Dev.	−3	−3	+5	+1
P. E.	2.5	1.5	2.5	1.5
Dev./P. E.	1.2	1.9	2.0	0.6

class does the deviation from expectation exceed twice the probable error. Brachyuric dwarfs could arise only from crossover gametes. Three such individuals were obtained where six would be expected if no linkage existed. This deviation is 1.9 times the probable error, but is less than the excess of normal normals, two-thirds of which would be expected to arise from crossover gametes. It is probable, therefore, that the deficiency of brachyuric dwarfs is due to lack of vitality in this doubly defective combination rather than to linkage. The class normal dwarf is close to the expected magnitude, though it is an exclusively non-crossover class and so should show considerable excess if linkage existed.

TABLE 3

TESTS FOR LINKAGE BETWEEN BRACHYURY AND HYDROCEPHALUS

	BRACHYURIC NORMAL	BRACHYURIC HYDROCEPHALUS	NORMAL NORMAL	NORMAL HYDROCEPHALUS
Observed	48	12	48	18
Expected	47.2	15.7	47.2	15.7
Dev./P. E.	0.2	1.5	0.2	0.9

A cross between brachyury and hydrocephalus gave even stronger evidence that these two genes are independent. See table 3. The agreement with expectation is in all cases excellent, the greatest deviation being observed in the doubly defective class, brachyuric hydrocephalus, which, like the brachyuric dwarf combination, is probably low in vitality. Nevertheless the deviation is here only 1.5 times the probable error and so not significant statistically.

Summary.—Linkage tests, involving the dominant lethal mutation brachyury (short tail) of the house mouse, show that it is independently inherited in relation to fourteen other mutant genes, among which no linkage is known to exist. It may be regarded as the marker of a fifteenth chromosome, unless linkage is subsequently found to exist among some of the fourteen test genes now thought to be independent.

[1] Dobrovolskaia-Zavadskaia, N., *Amer. Nat.*, **67**, 186–188 (1933).

[2] Dobrovolskaia-Zavadskaia, N., and Kobozieff, N., *C. R. Soc. Biol. Paris*, 1927–32.

[3] Chesley, Paul, *Proc. Soc. Exp. Biol.*, **29**, 437 (1932).

MIOCENE PATAGONIA

BY EDWARD W. BERRY

DEPARTMENT OF GEOLOGY, JOHNS HOPKINS UNIVERSITY

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In 1925 I described[1] 36 fossil plants from a locality named Mirhoja in Chubut Territory (Lat. 44° 20′ S., Long. 70° W.). These were considered to be Miocene in age because of certain similarities to the stratigraphically determined lower Miocene flora of Chile, which I had the pleasure of collecting from in 1929. The latter, described originally by Engelhardt[2] in 1891, is an extensive one containing well over 100 species. More recently a few Miocene, as well as earlier Tertiary plants from Argentina, made during the Willis Survey of 1911–1913, were determined[3] in the collections in the U. S. National Museum. These Miocene plants came from the Rio Chalia district of western Santa Cruz Territory Lat. circa 51° S., whereas the earlier Tertiary plants came from about 8 degrees farther north.

All of the collections of fossil plants from the Argentine which I have seen heretofore have been limited. Great interest attaches therefore to a very extensive collection which has recently come into my hands through the intelligent interest and coöperation of the collector Señor José Ramon Guinazú of the Direccion General de Minas y Geologia of the Republic of Argentina. This collection comes from the valley of the Rio Pichileufu, a permanent stream which has its source in the Andes south of Lago Nahuel Huapi and flows northward parallel with the Rio Limay for about 60 miles and finally empties into the latter. The exact locality is about 30 miles east of Lago Nahuel Huapi and about 360 miles almost due west from the port of San Antonio on the Gulf of San Matias, that is, about 41° 10′ south latitude and 70° 52′ west longitude. The altitude is between 900 and 1000 meters, and the country is a treeless pampa with an annual precipitation of less than 200 millimeters.

Pending the completion of the study of this material there are various facts which emerge and which it is desirable to place on record, particularly since the means for publication of elaborate studies scarcely exist any more.

The matrix is a fresh water fine-grained andesitic tuff. Aside from the plant remains there are a few cyprinodont fish scales and a sparing amount of rather poorly preserved insects—chiefly beetle elytra. The plants are represented almost entirely by leaves, chiefly of dicotyledons, although there are a few fruits and seeds, fragments of several different species of grasses and a variety of coniferous and fern types.

Fifteen of the 36 species described from Mirhoja have been recognized

in this new flora and what is more important in its bearing on the age of this flora as well as that from Mirhoja is that 23 species are common to the lower Miocene flora of Chile. There can scarcely be any doubt but that the age is lower Miocene.

One of the common elements in this flora is a species of Ginkgo, the first to be discovered on the continent of South America. It seems highly improbable that Ginkgo should have been an immigrant into South America in the Tertiary and this form is probably a descendant of an as yet undiscovered Mesozoic South American ancestry.

Three species of ferns are represented—an Anemia and 2 species of the family Cyatheaceae, both in fruit, and one almost certainly referable to the warm and wet climatic genus Balantium.

A species of the cycad genus Zamia is also common, as well as the coniferous genera Araucaria, Fitzroya, Libocedrus and Podocarpus.

The balance of the flora is prevailingly dicotyledonous and includes a number of genera not hitherto known as fossils such as the Proteaceous genus Guevina, the Flacourtiaceous genus Azara and the Eucryphiaceous genus Eucryphia. There is a characteristic species of Lomatia almost identical with the existing *Lomatia ferruginea* (Cav.) R. Br.

This flora is contemporaneous with at least a part of the extinct mammal fauna known as the Santa Cruz fauna and therefore afford a welcome addition to our knowledge of the environment of that curious fauna. Although a much more complete analysis of the flora will be required before one can speak with finality, the country during the lower Miocene was not as characteristically a semi-arid plains sort of a country as the students of the mammals have pictured. This conclusion was already foreshadowed by the much smaller flora from Mirhoja, but a single such occurrence might possibly be explained as a lakeside or riverside assemblage. Now that this same flora in reinforcements has been discovered far to the south of Mirhoja in the Rio Chalia region and to the north of Mirhoja in Rio Negro Territory and thus covers some 10 degrees of latitude, such an explanation becomes out of the question.

Furthermore the community between the contemporaneous wet flora of Chile and that of Patagonia proves that present conditions were not in existence during the lower Miocene and that at that time Patagonia was not in an Andean rain shadow, as it is at the present time.

A good many elements of this lower Miocene flora have the southern limit of their closest living relatives in southern Brazil, Paraguay and the Argentine Mesopotamia. There is a small element related to low altitude Bolivian Andean species and another element which persists at the present time in southern Chile and the Chilean Andes. The last is almost entirely coniferous and there are no traces of the Fagaceae which are so prominent in this flora at the present time. The Araucaria, represented by both

twigs and an abundance of cone scales, is closer to the modern Brazilian than it is to the Chilean species.

The flora is in no sense a rain forest, however, but a mixture of mesophytic and drier soil types. Thus there are a good many Sapindaceae, a fair number of Leguminosae and various other things indicating that while the rainfall was greater and better distributed and the temperature more genial than at present, the country was not completely forested. A more detailed analysis of the probable conditions is reserved for the final account of this flora. Another fact, already foreshadowed in earlier studies, is the lack of resemblance to African floras. The Miocene flora of Patagonia, as far as it is known, is typically American. This tends to discredit those students who would derive the Patagonian mammals from African ancestors, and it also furnishes no comfort to those who are inclined to believe in any of the current hypotheses of drifting continents.

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ON CERTAIN INVARIANTS OF CLOSED EXTREMALS

By Marston Morse and Everett Pitcher

Department of Mathematics, Harvard University

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Morse has classified closed extremals by what we shall call indices of periodicity. See reference 3, Ch. III. We propose in this paper to present briefly some facts about the determination of the indices of periodicity of a closed extremal and some necessary conditions on these indices in terms of a generalized Poincaré rotation number.

1. *Index of Periodicity.*—We are studying a calculus of variations problem in the usual parametric form with an integrand which is positive, of class C^3, homogeneous and positively regular. The space of the dependent variables is a regular, orientable, analytic $(n + 1)$-dimensional manifold. The non-orientable case is readily reducible to the orientable case.

We suppose there are closed extremals, of which g of length ω is one.

We first map g and its neighborhood on the x axis and its neighborhood in the Euclidean space $(x, y_1, \ldots, y_n)$ by a transformation with period ω in x. When the integral of our problem is transformed in the corresponding manner, the x axis is an extremal. We denote the segment $o \leqq x \leqq \omega$ of the x axis by γ.

We write the transformed integral in non-parametric form so that x is the variable of integration. The *Jacobi equations*, in the space (x, η), are then

$$\frac{d}{dx}\Omega_{\eta' i} - \Omega_{\eta i} = 0 \qquad (i = 1, \ldots, n). \tag{1}$$

See reference 3, Ch. I, ¶ 4. We are interested also in the *modified Jacobi equations*

$$\frac{d}{dx}\Omega_{\eta' i} - \Omega_{\eta i} + \lambda\eta_i = 0 \qquad (i = 1, \ldots, n). \tag{2}$$

See reference 3, Ch. II, ¶ 4.

The *index of periodicity* of g is the number of negative values of λ for which the modified Jacobi equations have a non-null periodic solution of period ω. We count a value of λ a number of times equal to the number of such solutions which are linearly dependent. See reference 3, Ch. III.

The *order of degeneracy* of g is the number of linearly independent solutions of the Jacobi equations which have the period ω. If the order of degeneracy is zero, we call the extremal g non-degenerate. *In this paper we shall suppose the extremal g is non-degenerate.*

A point $x = b$ on the axis of x is a conjugate point of the distinct point $x = a$ of index r if exactly r linearly independent solutions of the Jacobi equations vanish at $x = a$ and also at $x = b$. In counting conjugate points on an interval, we shall count each conjugate point a number of times equal to its index.

2 *Order of Concavity.*—Let G be the family of solutions

$$\eta_i = y_i(x) \qquad (i = 1, \ldots, n)$$

of the Jacobi equations joining those points in the n-planes $x = 0$ and $x = \omega$ whose coördinates (η) are the same. When g is non-degenerate, the solutions of G depend linearly on n linearly independent solutions of the Jacobi equations, which may be represented as the columns of the matrix

$$\|z_{ij}(x)\| \qquad (i, j = 1, \ldots, n).$$

Corresponding to a solution $\eta_i = \eta_i(x)$, $(i = 1, \ldots, n)$, of the Jacobi equations, we set

$$\Omega_{\eta' i}(\eta, \eta') = \zeta_i^{\eta}(x) \qquad (i = 1, \ldots, n) \tag{3}$$

The quadratic form*

$$K(u) = a_{\mu\nu}u_\mu u_\nu \qquad (\mu, \nu = 1, \ldots, n) \tag{4'}$$

in which

$$a_{\mu\nu} = z_{i\nu}(0)\,[\zeta_{i\mu}^{\varepsilon}(x)]_{x=0}^{x=\omega} \qquad (i, \mu, \nu = 1, \ldots n) \tag{4''}$$

is termed the *concavity form* of g. It is a symmetric form. Its index[5] and nullity[6] are independent of the matrix $||z_{ij}(x)||$ chosen as above. We term its index the *order of concavity* of g. Its nullity is the index of $x = \omega$ as a conjugate point of $x = 0$. See reference 3, Ch. III, ¶ 10, for a discussion of the concavity form.

If $x = \omega$ is not conjugate to $x = 0$, we may interpret the concavity form geometrically in the following manner. Let S be the manifold of points, in the space (x, η) of the solutions of the Jacobi equations, which lie on solutions belonging to G and passing through points (u) in the n-planes $x = 0$ and $x = \omega$ for which

$$u_1^2 + \ldots + u_n^2 = 1. \tag{5}$$

This manifold S is roughly a tube enclosing γ. The exterior normals to S at point (u) in the n-plane $x = 0$ and point (u) in the n-plane $x = \omega$ lie in a 2-plane through the x axis. Let θ be the angle between these normals, measuring θ from the normal at $x = 0$ and counting that sense of rotation positive which leads from the positive x axis to either normal. *Then for points on* (5) *the concavity form* $K(u)$ *and the angle* θ *satisfy the relation*

$$r(u) \sin \theta = K(u), \tag{6}$$

where $r(u)$ *is a positive continuous function of the variables* (u).

The following theorem is fundamental. See reference 3, Ch. III, ¶ 11.

THEOREM 1. *The index of periodicity of* g *is equal to the number of conjugate points of* $x = 0$ *on* γ *plus the order of concavity of* g.

The extremal g traced m times is a closed extremal. We shall denote it by g^m. We shall refer to the index of periodicity of g^m as the *mth index of periodicity* of g and denote it by T_m. We shall call the order of concavity of g^m the *mth order of concavity* of g.

We shall refer to the segment $0 \leqq x \leqq m\omega$ of the x axis as γ^m.

3. *Index of Repletion.*—If $m > 1$ and $x = \omega$ and $x = (m-1)\omega$ are not conjugate to $x = 0$, the matrices

$$||u_{ij}(x)|| \qquad ||w_{ij}(x)|| \qquad (i, j = 1, \ldots, n)$$

exist with columns which are unique solutions of the Jacobi equations satisfying the conditions†

$$\begin{aligned} u_{ij}(0) &= 0 & w_{ij}(0) &= \delta_i^j \\ u_{ij}(\omega) &= \delta_i^j & w_{ij}([m-1]\omega) &= 0 \end{aligned} \qquad (i, j = 1, \ldots, n).$$

Then we shall call the quadratic form

$$R(z) = [\zeta_{ij}^{u}(\omega) - \zeta_{ij}^{w}(0)]z_i z_j \qquad (i, j = 1, \ldots, n) \tag{7}$$

the *mth repletion form* of g and its index the *mth index of repletion* of g. Its nullity is the index of $x = m\omega$ as a conjugate point of $x = 0$.

The following theorem brings out the meaning of the indices of repletion.

THEOREM 2. *If $x = \omega$, $x = (m-1)\omega$, and $x = m\omega$ are not conjugate to $x = 0$, the number of conjugate points of $x = 0$ on γ^m exceeds the sum of the number on γ and the number on γ^{m-1} by the mth index of repletion of g.*

4. *Determination of Indices of Periodicity.*—We let

$$\|p_{ij}(x) \quad q_{ij}(x)\| \qquad (i, j = 1, \ldots, n),$$

whose columns are solutions of the Jacobi equations, be the base of solutions satisfying the initial conditions

$$\begin{aligned} p_{ij}(0) &= \delta_i^j & q_{ij}(0) &= 0 \\ \zeta_{ij}^p(0) &= 0 & \zeta_{ij}^q(0) &= \delta_i^j \end{aligned} \qquad (i, j = 1, \ldots, n).$$

We define the matrix A by the equation

$$A = \begin{Vmatrix} p_{ij}(\omega) & q_{ij}(\omega) \\ \zeta_{ij}^p(\omega) & \zeta_{ij}^q(\omega) \end{Vmatrix} \qquad (i, j = 1, \ldots, n). \tag{8}$$

We shall use the matrix A first in these two theorems.

THEOREM 3. *If for each positive integer m, g^m is non-degenerate and $x = m\omega$ is not conjugate to $x = 0$, then the mth order of concavity and the mth index of repletion of g are determined when A is known.*

THEOREM 4. *If for each positive integer m, g^m is non-degenerate and $x = m\omega$ is not conjugage to $x = 0$, then the mth index of periodicity of g is determined when A and the number of conjugate points of $x = 0$ on γ are known.*

We let W be a non-singular n-square matrix of constants. We set

$$\begin{aligned} P &= \|p_{ij}(\omega)\| & Q &= \|q_{ij}(\omega)\| \\ Z^p &= \|\zeta_{ij}^p(\omega)\| & Z^q &= \|\zeta_{ij}^q(\omega)\| \end{aligned} \qquad (i, j = 1, \ldots, n). \tag{9'}$$

We set‡

$$\begin{aligned} R &= WPW^{-1} & S &= WQW^* \\ Y^p &= W^{-1*}Z^pW^{-1} & Y^q &= W^{-1*}Z^qW^*. \end{aligned} \tag{9''}$$

We show under the conditions of Theorem 4 that the problem of determining the mth index of periodicity of g depends only on the number of conjugate points of $x = 0$ on γ and on invariants depending on the sets (R, S, Y^p, Y^q), where W is arbitrary subject to the conditions in the preceding paragraph.

5. *The Frequency Number μ.*—Any two solutions $\eta_i = v_i(x)$ and $\eta_i = z_i(x)$, $(i = 1, \ldots, n)$, of the Jacobi equations satisfy a relation

$$v_i(x)\zeta_i^q(x) - z_i(x)\zeta_i^p(x) \equiv \text{constant} \qquad (i = 1, \ldots, n)$$

and if this constant is zero, the two solutions will be termed *conjugate.* See reference 4, p. 626. There are at most n linearly independent solutions of the Jacobi equations in a set of mutually conjugate solutions. All the solutions which are linearly dependent on n mutually conjugate solutions will be said to form a *conjugate family,* and any set of n linearly independent solutions belonging to a conjugate family will be said to form a *conjugate base* of the conjugate family. A determinant whose columns are the solutions of a conjugate base will be termed a *focal determinant* of the conjugate family. The zeros of a focal determinant are isolated and of integral order from 1 to n. Any two focal determinants of a conjugate family vanish at the same points and to the same order. These points will be called *focal points* of the conjugate family. The order of vanishing of a focal determinant at a point is equal to its nullity at the point and will be referred to as the *order* of the focal point. In counting focal points of a conjugate family on an interval, each focal point will be counted a number of times equal to its order. This material is treated at length for a more general problem in reference 3, Ch. III.

Let F be a conjugate family with ν focal points on the interval $a < x \leqq a + m\omega$, $(m = 1, 2, \ldots)$. We define the frequency number $\mu(F,a)$ of g with respect to the conjugate family F and the initial point $x = a$ as the limit

$$\lim_{m=\infty} \frac{\nu}{m} \tag{10}$$

if this limit exists.

THEOREM 5. *The frequency number of g with respect to a conjugate family and an initial point exists and is independent of the conjugate family and the initial point.*

The number μ whose existence is affirmed in this theorem depends only on g and will be called the *frequency number* of g. The number $\frac{\omega}{\mu}$ is a generalization of the so-called Poincaré rotation number of a point transformation on a closed curve. See reference 7. Hedlund has shown the existence of this frequency number in the case $n = 1$ and has shown its relation to the problem of determining the indices of periodicity T_m in that case. See reference 8.

The following theorem gives a necessary condition on the indices of periodicity of g in terms of its frequency number.

THEOREM 6. *The limit*

$$\lim_{m=\infty} \frac{T_m}{m} \tag{11}$$

exists and is equal to the frequency number μ of g.

6. *Necessary Conditions on the Frequency Number.*—With the extremal

g we shall associate $2n$ numbers, $\rho_1, \ldots, \rho_{2n}$, which we shall call the *multipliers* of g. These numbers are the roots of the characteristic equation[9] of the matrix A.

There are no multipliers equal to 0. The non-degeneracy of g implies there are no multipliers equal to 1. We assume for convenience that there are no multipliers equal to -1.

The $2n$ numbers

$$\alpha_j = \delta_j + i\epsilon_j \qquad (j = 1, \ldots, 2n) \tag{12}$$

defined by the relations

$$\log \rho_j = \delta_j + i\epsilon_j \quad (-\pi < \epsilon_j \leqq \pi) \quad (j = 1, \ldots, 2n) \tag{13}$$

we shall term the *exponents* of g. Compare with reference 10.

Through application of the theory of lambda-matrices we have established these theorems.

THEOREM 7. *If none of the exponents of g is pure imaginary, the frequency number of g is an integer.*

THEOREM 8. *If the ratio of each pure imaginary exponent of g to $2\pi i$ is a rational number, the frequency number of g is rational.*

* We adopt the convention that a repeated subscript indicates summation with respect to that subscript.

† The symbol δ_i^j is 1 when $i = j$ and 0 when $i \neq j$.

‡ If B is a matrix, we denote its conjugate by B^* and its inverse by B^{-1}.

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CONFUSIONS IN THE USE OF THE MATHEMATICAL TERM GROUP

BY G. A. MILLER

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF ILLINOIS

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The technical term group is now employed with a variety of meanings even in our most commonly consulted works of reference, including the modern mathematical encyclopedias. For instance, near the beginning of the article entitled "Elementi della teoria dei gruppi" in the most recent of these encyclopedias, viz., the *Enciclopedia delle Matematiche Elementari*, volume 1, part 2 (1932), it is stated that the notion of group has been greatly extended during the last fifty years so as to include all those operations or transformations, finite or infinite in number, which may be performed upon the entities of a given field, whenever the transformation resulting from the successive application of any two of them belongs itself to these transformations. It should be noted that nothing is said here in regard to the laws according to which these transformations are combined and hence this extension of the notion of group tends to bring it into the realm of chaos, where it is impossible to make scientific progress.

It is evident that not a single one of the hundreds of theorems relating to abstract groups which have been published during the last fifty years could be proved if this extended notion of group were adopted, and hence one might at first be inclined to dismiss it from further consideration. While this method of procedure appears reasonable it fails to explain the fact that definitions which are in accord with this extended notion of group appear in many other places where one naturally expects to find reliable information. For instance, the term closed group (geschlossene Gruppe) is used for a set of numbers solely on account of the fact that it involves the product of every pair contained therein in the *Encyklopädie der Mathematischen Wissenschaften*, volume 1, page 652; and in volume 2, page 243, of the French edition of this work, a definition of the technical term group appears which is in complete accord with the extended notion of group to which we referred in the preceding paragraph.

Since the general concept of group is necessarily based on a system of postulates it is clear that this concept cannot be extended without modifying these postulates. The selected system should clearly be sufficiently restrictive to make it possible to base an extensive development thereon. While systems of postulates relating to finite groups were formulated by A. Cayley (1854) and H. Weber (1882), the latter formulated in 1893 a useful system which applies to groups of infinite order as well as to those of finite order. In particular, this system includes the now well-known equational

condition that when elements of a given group are substituted for any two of the three symbols in the equation $xy = z$ then the resulting linear equation has one and only one solution with respect to the elements of this group.[1] While this definition has been widely adopted it has not been sufficiently influential to prevent such vague uses of the term group as the one noted in the first paragraph of the present article.

It is perhaps especially noteworthy that in the article entitled "Endliche Discrete Gruppen" which appeared about five years later in the large German mathematical encyclopedia noted above a definition of the term group is given, volume 1, page 218, according to which a group does not necessarily involve either the identity or the inverse of each of its operators, as results from the fact that the totality of the natural numbers which exceeds an arbitrary one of them constitutes, according to this definition, a group with respect to multiplication. The totality of the natural numbers when thus combined is explicitly regarded as a group on page 43 of the article of the mathematical encyclopedia to which we referred in the first paragraph of the present article, as well as in many other places. This implies that a group does not necessarily involve the inverse of each of its elements. On the contrary, H. Poincaré and many others explicitly assume that the inverse of every substitution of a group appears therein, *Acta Mathematica*, **1**, 2 (1882).

Even some of those who do not believe that the concept of group has actually been extended during the last fifty years in the manner noted above may be interested in the facts which may have given rise to this view. While F. Klein (1849–1925) deserves much credit for creating a wide interest in the theory of groups and for exhibiting the usefulness of this theory, especially in geometry, he seems also largely responsible for some of the recent careless uses of the term group and may be largely to blame for the untenable view expressed by L. Berzolari in the Italian encyclopedia to which we referred in the first paragraph of this article. In 1884 he published his now famous *Vorlesungen über das Ikosaeder*, and on page 5 thereof he stated the following general definition: An arbitrary set of elements is said to constitute a group whenever the combination of every two of these elements belongs to the same set. It is true that he illustrates this definition by geometric elements which obey the other group theory laws when they are combined but he fails to direct attention to this fact in the definition. In a note to his well-known Erlanger Programm published in many places he directs attention to the supposed fact that it is not necessary that the inverse of every element appears in a group of infinite order and he gives here S. Lie the credit for having first directed attention to this difference between the elements of a group of finite order and those of an infinite order.

When such prominent workers in groups of infinite order, as F. Klein and

S. Lie were, stated that it is not necessary that such a group involves either the identity or the inverse of each of its operators, and when such statements are not modified in their recently published collected writings, it seems clear that the beginner in this field is exposed to serious misunderstandings unless these conditions are made generally known. It should be emphasized that according to some systems of postulates it is obvious that there are groups which do not involve the identity and the inverse of every element contained therein, while according to other such systems every possible group involves these elements. Hence there is no honor connected with the discovery of the existence of groups which involve neither the identity nor the inverse of each of their elements, although such honor might be inferred from some statements relating thereto. It is somewhat remarkable that F. Klein and S. Lie failed to adopt the abstract view as regards the definition of a group, especially since their contemporary H. Weber (1842–1913), who was born in the same year as the latter and was also very active in the field of group theory, had exhibited the advantages of this view.

It was noted above that according to the definition of the term group which appears in our largest modern mathematical encyclopedia the totality of the natural numbers which exceed an arbitrary given one of them constitutes a group with respect to ordinary multiplication. It should be added that these numbers also constitute a group with respect to ordinary addition according to the same definition. The undesirability of this definition is also exhibited by the fact that it has given rise to the view that a cyclic group of infinite order has two independent generators. This view was adopted by A. Loewy in his valuable article on algebraic group theory (Algebraische Gruppentheorie) published in the second edition, volume 1 (1910) of Pascal's *Repertorium der höheren Mathematik*, page 193. It is clear that if the powers of an element constitute a group then the exponents of these powers have the same property and hence it results that when s and t are of infinite order and satisfy the equation $st = 1$ they cannot be regarded as *independent* generators of a cyclic group, if we adopt the well-known definition of a group which involves the equational condition noted above, and appears in a simpler form on page 52, *Finite Groups* by Miller, Blichfeldt, Dickson, 1916.

It is easy to trace to their source the difficulties relating to the present definitions of the technical term group. This term was first employed with its modern meaning by E. Galois (1811–1832) with respect to substitutions on a given number of letters. Hence it related at first only to groups of finite order whose elements obey intrinsically all the conditions now imposed by the postulates on the elements of a group except the one which requires that the product of every two of the elements and the square of every one of them is again an element of the set. In various

other early developments of group theory the elements that were considered satisfy the same conditions and hence it resulted that the only postulate which was explicitly stated in some of the definitions of a group was the one which was not satisfied intrinsically by the elements under consideration. When the concept of group was later applied to more general elements and it became desirable to have a definition of the term group which applies to all of them such definitions were developed, but many writers continued to use the older definitions regardless of their inadequacy under the changed conditions.

The confusions resulting from conflicting definitions of the technical term group are augmented by inconsistencies and incompletenesses. While the French edition of the large mathematical encyclopedia contains the unusable definition noted above it also contains, with respect to groups of finite order, a satisfactory definition, volume 1, page 576. Both F. Klein and S. Lie sometimes assumed that the groups under consideration contain the inverse of each of their elements while at other times they directed attention to supposed groups which do not have this property. In the encyclopedia to which we referred in the first paragraph of the present article the definition of a group of infinite order is incomplete since it seems to assert merely that it is customary to add additional conditions without stating these definitely, page 33. Many of the definitions do not make it sufficiently clear that the square of every element is not necessarily included in a set of group elements which contains the product of every two distinct elements of the set. These PROCEEDINGS, **18**, 100 (1932).

[1] H. Weber, *Math. Annalen*, **43**, 522 (1893).

THE STRONTIUM II AND BARIUM II SPECTRA

BY F. A. SAUNDERS, E. G. SCHNEIDER AND EMILY BUCKINGHAM

RESEARCH LABORATORY OF PHYSICS, HARVARD UNIVERSITY

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The availability of pure metallic strontium and barium[1] has made it worth while to review their spectra, and especially to extend the observations of Lyman[2] in the Schumann region. We had available for this purpose a 1-meter vacuum grating spectrograph, using as source a direct-current vacuum arc between the metal and a graphite electrode. This work has been extended by using a similar source with quartz and glass prism spectrographs of varying dispersions, some of them quite large, and by studying the light from a hot quartz electrical furnace filled with metallic

vapor through which a high-voltage transformer discharge was sent, both with and without an external spark gap. From the large series of plates thus obtained, we have been able to obtain very completely the Ba I, Ba II, Sr I and Sr II spectra. The study of Ba II was rendered almost superfluous by the work of Rasmussen,[3] whose results were practically identical

TABLE 1

LINES AND TERMS IN Ba II

	λ	ν(VAC.)	TERMS	m
5D-mF	2347.58 (air)	42584.4	32427.7	4
	2335.25 (air)	42809.1	32203.0	
	2304.22 (air)	43385.5		
	1933.64 (vac.)	51715.9 (new)	23296.0	5
	1924.70 (vac.)	51956.2	23055.5	
	1904.15 (vac.)	52516.9		
	1697.16 (vac.)	58922.0 (new)	16091.0	6
	1694.37 (vac.)	59019.0	15990.1	
	1674.51 (vac.)	59719.0		
	1573.92 (vac.)	63535.6 (new)	11475.8	7
	1572.73 (vac.)	63583.5	11427.0	
	1554.38 (vac.)	64334.3		
	1504.01 (vac.)	66488.9	8545	8
	1486.72 (vac.)	67262.2	8517	
	1461.45 (vac.)	68425.2	6597	9
	1444.85 (vac.)	69211.3	6579	
	1433.15 (vac.)	69776.4 (new)	5250	10
	1417.14 (vac.)	70564.7 (new)	5237	
	1413.35 (vac.)	70753.9 (new)	4274	11
	1397.83 (vac.)	71539.4 (new)	4258	
5D-8P	1786.93 (vac.)	55961.3	19050.7	8P
	1771.03 (vac.)	56464.3	19351.6	
	1761.75 (vac.)	56761.7 (new)		
6S-8P$_{1/2}$	1630.40 (vac.)	61334.6		
4F-mG	3472.71 (air)	28787.7	3639.9	10G
	3430.18 (air)	29144.7	3058.4	11G
6P-9D	2054.57 (air)	48671.8 (new)	10066.0	9D
	2052.68 (air)	48701.1	10034.0	
	1985.60 (vac.)	50362.7		
6D-7P	2023.95 (air)	49392.5	31296.0	7P
			30675.1	
6P-10S	2009.20 (air)	49754.9	10670.8	10S
6S-7P	1998.87 (vac.)	50012.0		
6P-11S	1970.19 (vac.)	50756.4 (new)	7981.6	11S
6P-10D	1954.21 (vac.)	51171.6 (new)	7589.5	10D
	1892.65 (vac.)	52836.0 (new)	7563.0	
7S-7P	13057.4 (air)	7656.7 (Randall)		
	14211.4 (air)	7034.9 (Randall)		

with our own. We have, however, extended his 5D-mF series by two more terms and three new "satellites," which are presented in table 1 together with a few other new lines and terms. The terms of Ba II as corrected by Rasmussen are checked by our work and need no modification. We present new wave-lengths for most of the lines below 2000 A. U., measured from recent standards.

The interest in the Ba II spectrum lies in two anomalies, which are unusual, if not unique, in one-electron spectra. Unlike Ca II and Sr II, Ba II shows a reversal of relative intensity in the principal series pairs 6S-7P and 6S-8P, as compared with 6S-6P. Thus λ 4554 is more intense than λ 4934, but λ 2024 is much more intense than λ 1999. The same intensity relations appear in 7S-7P, compared with 6P-7S. In the term 6P, the more populated level is the one for which $j = {}^3/_2$; in 7P it is the one for which $j = {}^1/_2$. The intensities alone are inverted, not the positions.

The other anomaly in Ba II is the faintness and odd separation in the group 5D-5F (see table 1). The line at λ 1933 was found only after many attempts. The table shows the abnormal term separation here, as noted by Rasmussen. Some sort of perturbation evidently occurs here, but it appears to be of a novel type.

TABLE 2

SR II SPECTRUM

	λ	ν(VAC.)	TERMS	
5S-5P	4215.52	23715.4	65248.6	5P
	4077.71	24516.8	64447.2	
5S-6P	1793.10 (vac.)	55769.3 (new)	33194.3	6P
	1783.97 (vac.)	56054.3 (new)	32906.1	
5P-6S	4305.46	23219.9	88964.0	5S
	4161.81	24021.4	41227.3	6S
5P-7S	2471.63	40447.2	23999.9	7S
	2423.53	41248.9		
5P-8S	2052.54 (vac.)	48720.1	15726.9	8S
	2019.31 (vac.)	49522.0		
5P-9S	1874.90 (vac.)	53336.2 (new)	11106.4	9S
	1846.76 (vac.)	54148.8 (new)		
5P-5D	3464.47	28856.3	74408.0	4D
	3474.90	28769.7	74127.6	
	3380.72	29571.2	35677.4	5D
			35590.9	
5P-6D	2324.52	43006.7	21441.0	6D
	2322.39	43046.1	21401.1	
	2282.02	43807.2		
5P-7D	1995.78 (vac.)	50105.7 (new)	14342.7	7D
	1995.00 (vac.)	50125.3	14320.9	
	1964.43	50905.3		

TABLE 2 (*continued*)

	λ	ν(VAC.)	TERMS	
5P-8D	1845.45 (vac.)	54187.3	10275.2	8D
	1819.01 (vac.)	54974.9	10261.6	
5P-9D	1762.81	56727.6 (new)		
4D-4F	2165.93 (air)	46155.1	27972.3	4F
	2152.84 (air)	46435.8		
4D-5F	1778.39 (vac.)	56230.6	17898.2	5F
	1769.63 (vac.)	56509.0		
4D-6F	1620.35	61715.1	12410.6	6F
	1612.98	61997.0		
4D-7F	1537.91	65023.3 (new)	9102.7	7F
	1531.28	65305.0 (new)		
4D-8F	1488.99	67159.0 (new)	6958.1	8F
	1482.69	67444.1 (new)		
4D-5P	10914.8 (Meggers)	9159.3		
	10327.3	9680.4		
	10036.6	9960.8		
4D-6P	2425.62 (air)	41214.0 (new)		
	2425.17 (air)	41221.6 (new)		
5D-7P	6509.20	15358.6 (new)	20318.8	7P
	6483.17	15420.3 (new)	20170.4	
5D-8P	4556.89	21938.7 (new)	13652.2	$8P_{3/2}$
4F-4G	9644.2	10366.1 (new)	17606.2	4G
4F-5G	6349.00	15746.1 (new)	12226.3	5G
4F-6G	5263.73	18992.6 (new)	8979.7	6G
4F-7G	4738.30	21098.7 (new)	6873.6	7G
4F-8G	(Ca. coincides)	(22542.1)	(5430.2)	8G
4F-9G	4240.54	23575.3 (new)	4397.0	9G
4F-10G	4107.55	24338.6 (new)	3633.7	10G
6S-6P	12445	8033.1 (Randall)		
	12016	8320.0		
6P-8S	5819.00	17180.3 (new)		
	5723.70	17466.3 (new)		
6P-9S	4585.91	21799.8 (new)		
	4526.10	22087.8 (new)		
6P-10S	4056.67	24643.8 (new)	8262.2	10S
	4009.75	24932.2 (new)		
6P-11S	3769.99	26517.8 (new)	6387.9	11S
	3729.34	26806.8 (new)		
6P-12S	3593.23	27822.2 (new)	5084.1	12S
	3556.44	28110.0 (new)		
6P-13S	.....	(28761)	4144.9	13S
	3441.43	29049.4 (new)		

6P-7D	5385.45	18563.4 (new)		
	5379.13	18585.2 (new)		
	5303.13	18851.5 (new)		
6P-8D	4417.50	22630.9 (new)		
	4414.84	22644.5 (new)		
	.....	(22920)		
6P-9D	.....	(25182)	7723.8	9D
	(Ca. coincides)	(25191.1)	(7715.0)	
	3925.00	25470.5 (new)		
6P-10D	3717.30	26993.6 (new)	6012.5	10D
	3677.56	27184.2 (new)	6010.1	
6P-11D	3559.10	28088.7 (new)	4822.0	11D
	3523.51	28372.3 (new)	4817.1	
6P-12D	3452.30	28957.9 (new)	3948.5	12D
	3418.36	29245.4 (new)		
6P-13D	3376.35	29609.3 (new)	3295.2	13D
	3343.46	29900.6 (new)		
6P-14D	.....	.....	2789.4	14D
	3288.00	30404.9 (new)		
5D-4F	13124	7611.8 (Randall)		
	12974	7705.6 (Randall)		
5D-5F	5650.54	17692.5 (new)		
	5622.94	17779.3 (new)		
5D-6F	4312.74	23180.6 (new)		
	4296.82	23266.5 (new)		
5D-7F	3774.22	26488.0 (new)		
	3762.00	26574.1 (new)		
5D-8F	3491.62	28631.8 (new)		
	3480.97	28719.4 (new)		
5D-9F	3321.34	30099.7 (new)	5491.3	9F
	3311.85	30185.9 (new)		
5D-10F	3209.56	31147.9 (new)	4443.0	10F
	3200.68	31234.4 (new)		
5D-11F	3131.75	31921.8 (new)	3669.2	11F
	3123.30	32008.2 (new)		
5D-12F	3075.01	32510.8 (new)	3080.1	12F

The Sr II spectrum is regular in every way and similar to that of Ca II. We have found the 4F-mG series, and from these lines, which follow a simple formula, we have calculated the term 4F, and find that its value (and the values of all other terms as given in Bacher & Goudsmit, "Atomic Energy States") must be raised by 12 units. We have also obtained members of the following series, 4D-mP, 5D-mP, 6S-mP, 6P-mS, 6P-mD and 5D-mF, using in addition to our own observations others in the ultra-red kindly communicated by Professor H. M. Randall.

The results for Sr II are set forth in table 2, the notation being that of Bacher and Goudsmit.

[1] We are much indebted to Dr. A. J. King of Syracuse University for his kindness in sending us this material.

[2] T. Lyman, *Astrophys. Jour.*, **35**, 341 (1912).

[3] E. Rasmussen, *Zeits. Physik*, **83**, 404 (1933).

THE EFFECT OF INTENSITY AND WAVE-LENGTH ON THE RESPONSE OF AVENA TO LIGHT

By Charles Haig

Laboratory of Biophysics, Columbia University

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1. *Orientation to Light.*—When the young seedling of the oat (*Avena*) is exposed to unilateral illumination of moderate intensity, it bends toward the light. The explanation of this response is based on the fact that *Avena* is one of the many plants which elongate more rapidly when grown in the dark than in the light.[1,2] Thus, when *Avena* is illuminated from one side only, the coleoptile bends toward the light because the proximal side grows more slowly than the distal side.[3] In the case of plants like *Phymomyces*, whose longitudinal growth is more rapid in the light than in the dark, unilateral illumination causes positive bending because the optical characteristics of the shoot are such that the light is focused on the far side.[4,5]

Until recently, Darwin's[6] conclusion that the receptors involved in photogrowth and phototropic responses are confined to the first few millimeters or *tip* of the coleoptile was generally accepted. But in 1925 Bakker[7] found in the unilaterally illuminated shoot of *Panicum* of which the cotyledon (*tip*) is shielded from the light by a cylinder of tin-foil, that the hypocotyl (*base*) bends and the cotyledon remains unaffected. Very likely there are thus two responses in the naked plant, but normally the response of the cotyledon masks the response of the hypocotyl. F. W. Went's[8] experiments with *Avena* indicate a similar independence of the tip and base portions of the coleoptile. He found that a generally illuminated plant shows two periods of growth inhibition, one beginning 8 minutes and the other 28 minutes after stimulation. When all but 3 mm. of the tip is shielded, only the 28 minute response occurs, and when about 1.25 mm. of the tip is shielded, only the 8 minute response appears. These two responses are probably related to the two positive reactions (separated by a negative one) found by Arisz[9] with increasing light energy, and to the growth inhibitions reported by van Dillewijn[3] which occur under the same conditions.

The present work was undertaken to secure a precise description of this

situation by means of a quantitative study of the bending responses of *Avena*, when naked and when shielded, to light of different intensities and spectral composition.

2. *Procedure.*—Etiolated seedlings of *Avena sativa* 8 to 12 mm. long were used. Throughout each experiment from germination onward the temperature of the seedling was maintained at 25° C. by means of an air thermostat. A beam of parallel light rays from a 500-watt concentrated-filated lamp was made to fall on one side of the plant. The intensity of

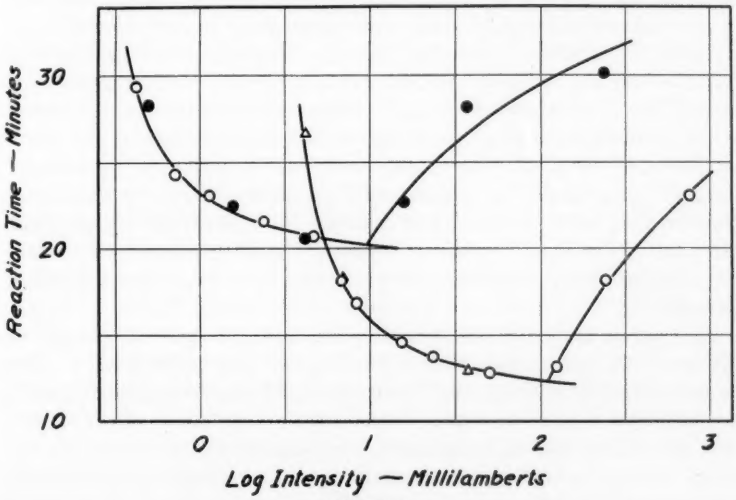

FIGURE 1

Relation between reaction time and intensity of white light falling on one side of *Avena* for 1 second. Each point is the average of 10 single readings, each on a different plant. Open circles: naked coleoptiles. Solid circles: bases shielded. Triangles: tips shielded. Descending curves are hyperbolas derived from the data as described in the text. Ascending curves are computed on the basis of a delaying effect at high intensities.

the light was varied by properly placed diaphragms, and its spectral composition was controlled by color filters. With white light the plants were exposed for one second by means of a camera shutter. For the colored lights some exposures were for 10 and 30 seconds and were controlled by a stop-watch. The position of the apex of the shoot was followed by a telescope which was displaced horizontally by a micrometer screw. During each measurement the plant was illuminated by a dim red light to which it is insensitive. The position was recorded every minute, beginning a few minutes before the exposure and continuing well beyond the time when bending had become established. The positions were then plotted against

time, and the moment for the onset of bending determined graphically to the nearest minute. The time between the beginning of the exposure and the beginning of the bending is here called the reaction time.

3. *Reaction Time and Intensity of White Light.*—For finding the relation between the intensity of the light and the reaction time, I made ten determinations, one with each of ten separate plants, of the time of the onset of bending at each of thirteen intensities. The exposure was always one second. The open circles in figure 1 show the average data. It will be noted that as the stimulating intensity increases, the reaction time falls in two distinct and regular steps from the neighborhood of 30 minutes to about 12 minutes and then rises steeply. The double relation between reaction time and intensity secured here is very likely an expression of the tip and base growth response found by Went. At 500 meter-candle-seconds Went obtained a double growth-reaction from the naked plant, one after 8 minutes and the other after 28 minutes. At this intensity ($\log I = 1.7$, since 10 meter candles is almost equal to one millilambert) the average reaction time for my plants is 13 minutes, only the earlier response being recorded. My efforts to observe a second bending yielded indifferent results because of the complications introduced by the restorative geotropic response.

As a purely empirical description of the data, the relation between the reaction time and the logarithm of the intensity (up to the point of sharp increase in reaction time) may be represented by a rectangular hyperbola $(r-m)(\log I + a) = k$, where m and a are the asymptotes of the reaction time and the $\log I$ axes, respectively, r is the reaction time and k is a constant. The two descending curves in figure 1 are such hyperbolas, the equation for the upper data being $(r-18)(\log I + 0.7) = 3.75$, and that for the lower being $(r-10.2)(\log I - 0.41) = 3.35$. It is apparent that the data are very precisely described by these curves. All the measurements of reaction time against $\log I$, which I have made, follow such a rectangular hyperbola, as will be apparent in the course of this paper.

4. *Reversal at High Intensities.*—At intensities above 30 times the threshold intensity the reaction time rapidly increases. This is true for both parts of the data, the data for the upper curve (solid circles) having been secured by a method described in the next section. All the factors involved in this increase are not known, but some understanding of the situation at high intensities may be obtained from the following considerations.

Van Dillewijn[3] has shown that about $^1/_{30}$ of the light falling on the proximal side of the plant passes through to the distal side. At low intensities this transmitted light will be below the threshold value for photo-growth reaction of the distal side; but as the intensity is increased, this side will finally also receive enough light for effective inhibition of its growth. At

intensities above 30 times the threshold intensity, one would thus expect a positive response of short reaction time followed by a retardation of the bending as the distal response begins. The plant, however, does not behave in this way. Instead the reaction time is lengthened, and the higher the intensity the longer it becomes. After the bending response has proceeded for some minutes, retardation suddenly begins and tends to cause the plant to bend away from the light.

It would thus appear that a concentration of photoproducts on the far side, so small that the resulting response would occur only at the end of 20 or 30 minutes, is nevertheless able to delay the onset of positive bending which normally would occur in 13 minutes. Assuming that the final reaction velocity $1/(r-m)$ at a given illumination is proportional directly to the proximal light effect and inversely to the distal effect, and that the distal intensity is $^1/_{34}$ of the proximal intensity, it is possible to describe the data for the high intensities. The two ascending curves of figure 1 were computed in this manner. The agreement with the base responses could hardly be better, while the largest deviations from the tip data are about equal to the probable errors.

5. *Anatomical Separation by Shielding.*—If the double relation between reaction time and intensity shown in figure 1 represents the separate sensitivities of the base and the tip of the plant, it should be possible to separate the two by mechanically confining the illumination to one or the other of the two parts of the plant. Experiments showed this supposition to be correct. The base was shielded by placing a tube of tin-foil around all but 1.5 mm. of the tip, and the tip was shielded by dipping 3 mm. of it in a suspension of lampblack until the dried coating was quite opaque. Otherwise the exposure and measurements were made as before.

The solid circles of figure 1 represent readings obtained by illumination of the tip only, and the triangles those resulting from stimulation of the base alone. Each point is the average of ten measurements. It is apparent that the measurements made with partial illumination not only fall on the two curves calculated from the data on naked plants, but yield data quite unobtainable except by a shielding procedure. Whereas with naked plants the upper curve could not be extended beyond the point at which the lower curve begins, with plants whose base is shielded this is possible. What is more, the data so secured are in fair agreement with the ascending theoretical curve computed as previously described. It will be noted that the reaction time at 500 M.C.S. ($\log I = 1.7$) is almost exactly the value, 28 minutes, obtained by Went for the tip growth response at this intensity. Furthermore, the reading at 26.7 minutes ($\log I = 0.62$) on the lower curve of figure 1 could not be made if the tip were exposed to the stimulating light.

6. *Separation by Color Filters.*—The data seem to provide clear evidence

that there are two photoreceptor systems with separate loci in the tip and base of the coleoptile, and that these two systems function at different intensity levels. On the chance that they may also differ in regard to spectral sensibility, an attempt was made to separate them by the use of color filters. For this purpose Wratten filters (Eastman Kodak Company "Monochromatic"), were placed in the path of the light beam at a point near the plant. For violet light monochromatic filter No. 76 (center of gravity at λ 4485) was used, and for blue-green light monochromatic filter

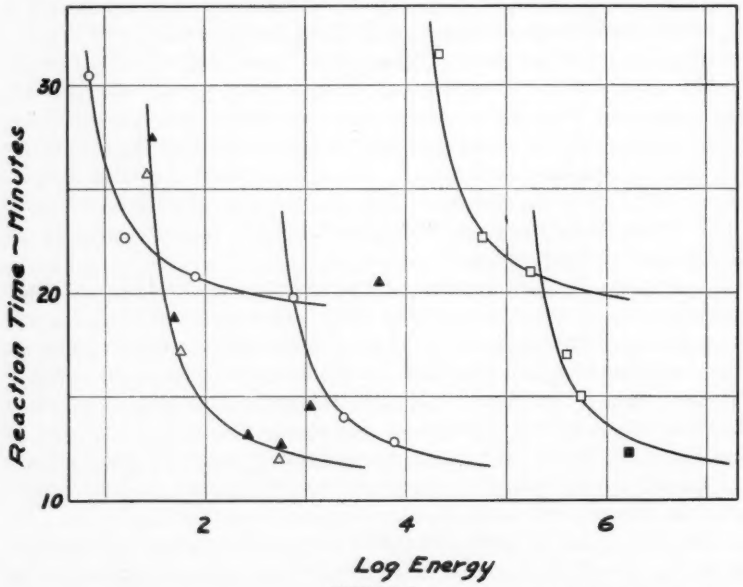

FIGURE 2

Reaction time-energy curves for responses to three regions of the spectrum transmitted by three different filters. Each point is the average of 6 single readings, each on a different plant. *Blue-green:* open circles, 1 second exposure. *Violet:* open triangles, 1 second; solid triangles, 10 seconds. *Minus-blue:* open squares, 10 seconds; solid squares, 30 seconds.

No. 75 (center of gravity at λ 4905). It soon became apparent that the plant was so insensitive to wave-lengths above those transmitted by filter No. 75, that only a minus-blue filter (No. 12) which transmits all wave-lengths above λ 5000 could be used without substituting a more powerful source. The experimental procedure was exactly as described for the study of naked plants stimulated by white light.

Figure 2 presents graphically the averaged data for the three filters. The abscissas are in relative energy units determined from measurements

of the energy distribution in the lamp and the transmissions of the filters.

The filters absorbed such large quantities of light that it was found necessary to increase the exposure time to 10 seconds, and in one case to 30 seconds, in order to obtain sufficient energy to complete the curves. This involves the assumption that the Bunsen-Roscoe relation holds for *Avena*. Justification for this assumption is supplied by the work of Blaauw,[10] who found that for a given bending response of this plant the product of intensity and time is a constant over enormous ranges of intensity and time. But in order to be quite sure that the procedure was sound, complete 1 and 10 second exposure curves were obtained for violet light (filter 76). It will be seen from figure 2 that the 10 second readings (solid triangles) agree very well with those obtained by 1 second exposures (open triangles).

Inspection of figure 2 reveals that no tip curve could be obtained when the violet filter was used, indicating that in the spectral region transmitted by this filter the base responses have lower thresholds than the tip responses. Such a filter thus affords a means of separating the two types of response without shielding. On the other hand it is clear that in the blue-green and minus-blue regions the tip is more sensitive than the base, as is the case when white light is the stimulus.

As before, all the curves are drawn as rectangular hyperbolas, and it is apparent that they adequately describe the data. The three base curves may be superimposed by shifting them along the abscissa. In the same manner, the tip curves may be superimposed. By determining the amount of displacement necessary in each case, we have a measure of the relative effectiveness of the three kinds of light. It turns out that whereas the base is 1000 times more sensitive to blue-green and 18,000 times more sensitive to violet than to minus-blue light, the tip is 3000 times more sensitive to blue-green than to minus-blue. These differential sensibilities to color are sufficient to show a considerable difference between the slopes of the tip and base spectral sensibility curves included in these wavelengths; the tip curve having a greater slope. The data also indicate a maximum sensibility in the violet or ultra-violet for the base response, and in the blue for the tip response.

7. *Theoretical.*—The simple, reciprocal relation found in all my measurements between log I and reaction time suggests certain theoretical possibilities. According to Blaauw, bending is due to a differential growth-rate of the two sides of the plant. The effect of momentary unilateral illumination may thus be to destroy or otherwise inactivate the growth process (*Auxin?*)[11,12,13,14] on the illuminated side, slowing up growth on this side and thus causing the coleoptile to bend toward the light. The shorter the reaction time, the faster must be the process leading up to the inhibition of growth. Hence, the reciprocal of the reaction time is a mea-

sure of the speed of this preliminary process. The reaction time apparently has a time limit m below which it never goes. A plot of $1/(r-m)$ against log I gives a straight line, indicating a direct proportionality between log I and the speed of the process occurring in the interval $r-m$. This is expected from the hyperbolic relation between log I and $r-m$. An identical relation between this period and log I has been found for the photic responses of *Mya* and *Pholas* (Hecht[15,16]), and a similar relation is indicated by the work of Castle[4] on *Phycomyces*. The constant interval m is probably occupied by extra-receptor preparations for bending.

We may suppose that there are photo-labile substances in the shoot that are decomposed by light during the very brief exposure period. Their decomposition products determine the speed of a secondary non-photic reaction whose products inhibit growth. If we assume that the velocity of the second reaction is directly proportional to the concentration of photoproducts, then the fact that $1/(r-m)$ plotted against log I yields a straight line shows the concentration of photoproducts to be a logarithmic function of the intensity, a relationship common in reversible photochemical systems possessing stationary states. Apparently there are two such sensitive systems present in the plant.

8. *Summary.*—The reaction time for positive phototropic responses of *Avena sativa* for short exposures to white light decreases with increasing intensity up to about 100 ml. and then increases. The response curve shows two parts, indicating a double photoreceptor process. The two response curves are rectangular hyperbolas, and the speed of the reaction time process is a logarithmic function of the intensity.

When only the first 1.5 mm. of the tip of the coleoptile is stimulated, only one response curve is obtained. When all but the upper 3 mm. of coleoptile is exposed, the other single curve is obtained. Hence, the two photoreceptor systems have separate loci.

The relative sensibility of the tip of the shoot to lights of three different colors is not the same as that of the base. This suggests that the two sensitive regions possess qualitatively distinct photochemical systems.

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TEMPERATURE COEFFICIENT OF NERVE DEGENERATION

BY T. W. TORREY

DEPARTMENT OF ZOÖLOGY, INDIANA UNIVERSITY

Communicated March 23, 1934

When a nerve trunk is cut or is killed at any point by crushing or other means, all the fibers peripheral to the point of injury undergo degeneration. The time required for the process to reach a given end-point differs, first, for the several kinds of fibers found in the animal body and, second, for varying temperatures. It has long been known that the nerves of warm-blooded animals will disintegrate much earlier and with greater rapidity than those of cold-blooded forms. Too little attention, however, has been paid to the governing factor of temperature, with the result that the literature contains numerous conflicting accounts of the histological picture of a degenerating nerve at the end of a particular period of time. In most cases the observations of each of the disagreeing investigators have been correct, the differences resulting from a failure to take into account the variations in temperature to which the nerves have been subjected. This applies primarily, of course, to those experiments carried out on cold-blooded animals.

Specific observations on the precise relationship between speed of degeneration and a recorded change in temperature have not been many and for the most part have been rather casual. Mönckeberg and Bethe ('99) found that to reach a certain stage in the degeneration process—the formation of ellipses—required something like only a third as long a time in frogs operated on in the summer as in those similarly treated in winter. The same condition could be seen in rabbits even sooner than in the "summer" frog.

Merzbacher ('03) made a comparison of results in the bat when the animal was hibernating and when kept awake. There was a delay in nerve degeneration of several weeks in the hibernating animal, a delay rightly interpreted to be the result of a lower temperature and not a property of hibernation itself. Further experiments by Merzbacher on frogs kept at different temperatures confirmed these results.

More recently (Rindovie, '25) the temperature coefficient of degeneration has been calculated and for the interval between 14°C. and 24°C. has been

found to be 2.2. It is of interest that this figure happens to be exactly that of the respiratory rate. (Gerard, '27.)

This value is close to that expected from Van't Hoff's rule, but, it must be noted, concerns only a restricted interval in the middle region of the temperature scale. In general the Q_{10} ratio shows a distinct falling off at high temperature levels, the ideal value of 2–3 holding only between 15° and 30°. It seems worth while, therefore, to record values for intervals other than the one mentioned.

The tibialis nerve in the leg of the frog, Rana pipiens, was utilized for the experiments. The nerves in both legs of an individual were severed at the level of the knees, the animal placed in a vessel of shallow water maintained at a chosen temperature, and the desired number of days allowed to elapse. The use of both limbs served to double the amount of material for examination. So as to guard against the precocious degeneration near the wound, the segments to be examined were removed near the middle of the peripheral strand and at the same level on both sides. The possible error induced by progressive degeneration in the nerve was thus also avoided. The strands of removed nerve were fixed and stained with osmic acid and sectioned longitudinally.

Assuming that an increase of 10° in temperature would approximately double the rate of degeneration, the following interpretation should be sound. A nerve allowed to degenerate for ten days, say at 15°C., should present about the same histological picture as one which had run five days at 25°C. Further refinement would consist in obtaining nine-, ten- and eleven-day preparations at 10° to compare with the five-day-25° preparation. Half-day intervals would be an additional refinement.

If a ten-day histological picture and a five-day picture presented the same appearance, the Q_{10} would, of course, be 2. If, however, *eleven* days and five were identical, the Q_{10} would be 2.2. Expressed in other terms, at 25° less than half the time is required for a nerve to reach a given state of degeneration than is necessary for the same state to be attained when the temperature is ten degrees lower.

Utilizing this method of comparison, the temperature coefficients for several levels have been obtained. The figures are assembled in the following table, each representing an average of six calculations. The maximum individual variation was in no case more than 0.1.

TEMPERATURE INTERVAL	Q_{10}
22–32°	1.6
15–25°	2.2
12–22°	2.8
9–19°	3.5

The above values have an important significance in addition to the mere increase in the list of known values for nerve degeneration.

It has been pointed out previously (Torrey, '31) that the usual chemical activity of nerve must be thought of as persisting even during degeneration. Any such chemical reaction presupposes the existence of the necessary reacting materials and implies, in addition, the utilization of oxygen and evolution of carbon dioxide. Both the latter features have repeatedly been observed. That degeneration is itself primarily a chemical process is further suggested by the fact that Q_{10} values of 2–3 are indicative of chemical rather than physical reactions.

Why a severed nerve should degenerate at all so long as its blood supply is intact can be explained only upon the basis of an elimination of some sort of a trophic influence of the cell-body over the peripheral element. Inasmuch as the functional life of a severed nerve fibre may be directly influenced by temperature (or other modifying factors, e.g., electrical stimulation), it follows that such an influence must be directed against some physiological or nutritional requirement of the fibre. It seems likely, therefore, that this requirement, or trophic influence of the cell-body, as you will, is of the nature of a nutrient material which in some fashion makes its way from the cell-body along the nerve fibre.

It is of interest that the Q_{10} values obtained for the degenerating frog nerve check fairly closely with a corresponding set of values for degenerating taste-buds. (Torrey, '34.) Evidence has been presented in this latter connection to show that the nutrient material, thought to be necessary for the existence of nerve fibres, is likewise necessary for the maintenance of such related sense organs.

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THE PROLONGED ACTIVITY OF MOMENTARILY STIMULATED NERVES

BY G. H. PARKER

ZOÖLOGICAL LABORATORY, HARVARD UNIVERSITY

Read before the Academy, Monday, April 23, 1934

According to conventional neuro-physiology a nerve when severed from its center and momentarily stimulated is supposed to respond by momentary activity as is well shown in the single twitch of the attached muscle when a severed motor nerve is subjected to a single electric shock. Such momentary activity does not seem to characterize chromatophoral nerves, for, when cut, they remain active as a result of the severance for many hours or even days. This condition is illustrated in the responses of the melanophore nerves in the caudal fins of many bony-fishes.

If one or more bony rays in the tail-fin of a catfish (Ameiurus) or of a killifish (Fundulus) are completely severed near the root of the tail together with their accompanying nerves, the melanophores in the radial band thus denervated pass quickly into a state of dispersed pigment whereby the band becomes noticeably dark (Fig. 1). This dark band reaches a maximum depth of tint in a few hours and then gradually fades till it attains the general tint of the fish in from a day or so to a week. The continuation of this state over so long a period is believed to be due to the continued activity of the severed nerve-fibres.

Instead of the interpretation just given it might be assumed that the nerves whose severance brings about a dispersion of the melanophore pigment exert an inhibitory action on this response and that when they are cut the melanophores react as to a release from this inhibition. But this view can be shown not to be sound by what is to be observed when a cold-block is applied to the nerve. Drugs cannot well be employed for this purpose for the following reasons. The radial nerves in the tail-fin of the killifish are at best not over a centimeter and a half long and any drug applied to the middle of such a nerve quickly spreads along its length and may thus directly affect the associated melanophores. Cold, however, can be used as a block with full satisfaction.

If the tail of a living killifish is spread out on a white board and a bent capillary glass tube is applied for about two millimeters of length transversely to its rays and on the middle of their length, a cold fluid (50 per cent alcohol) can be run through the tube so as to chill the nerve to a few degrees below freezing. In the actual tests the cold alcohol mixture left the reservoir at about $-13°$C. and emerged after its passage over the nerve at from $-1°$ to $-2°$C.

When radial nerves are thus chilled impulses causing the dispersion of

melanophore pigment are blocked. This can be shown by cutting such nerves proximal to the chilled area. On carrying out such an operation the denervated band quickly darkens from the cut to the chilled area which, however, the dark band neither enters nor passes. If the nerves distal to the chilled area are cut the distal region darkens from the new cut to the edge of the fin (Fig. 2). These observations show that the nerves both proximal and distal to the chilled area are normally active and, what is equally important, that the chilled area is a complete block for impulses concerned with melanophore changes. If on thus blocking these impulses the distal melanophores show no change, as is the case, we are warranted in believing that the impulses that naturally pass over these fibres are not inhibitory impulses but those concerned with positive excitation.

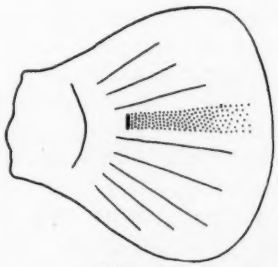

FIGURE 1

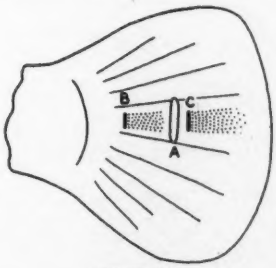

FIGURE 2

Figure 1.—Caudal fin of a killifish (Fundulus) showing a band of dark melanophores produced by the severance of radial nerves near the root of the tail.

Figure 2.—Caudal fin of a killifish across a part of which a capillary tube (*A*) carrying a freezing mixture has been placed. The cut (*B*) proximal to the tube has been followed by the formation of a dark band which reaches from the cut to the chilled area but does not enter it; the cut (*C*) distal to the tube has been followed by the formation of a band which reaches to the edge of the fin.

It might be supposed, however, that the quick darkening in the formation of the bands and their slow fading is a reflection of gradual degeneration in the nerve-fibres, a phenomenon that is well known to occur in these very nerves (Parker and Porter, 1933). But if a band is allowed to fade, which may occur, as already stated, in a day or more, and a new and shorter cut is made within the area of the band and distal to the original one, a smaller but well defined band within the limits of the first will quickly develop (Fig. 3). This shows at once that the nerves are still active and have not yet suffered even the early stages of degeneration. The appearance and disappearance of the band, therefore, is not to be attributed to degeneration.

It might be assumed since the exciting cut severs small bony rays and

since the tail is in more or less continual movement that these cut rays rub the severed ends of the nerves and thus continually excite them to action till this condition is overcome by eventual healing. If, however, in place of a simple transverse cut in the tail, a square hole is made in it so that the ends of the cut rays cannot rub against the nerves, this possible source of stimulation can thus be removed. Under such circumstances the dark band forms as quickly and as fully as it does from the simple transverse cut (Fig. 4). The rubbing of the cut fin-rays on the severed ends of the nerves then appears to play no essential part in the activation of the melanophore nerves.

In consequence of these several observations I am led to conclude that the nerve-fibres concerned with the dispersion of melanophore pigment even when separated by severance from their nerve centers, continue to

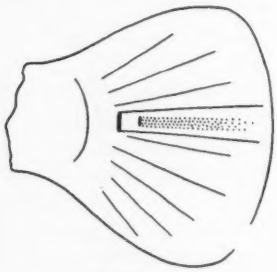

FIGURE 3

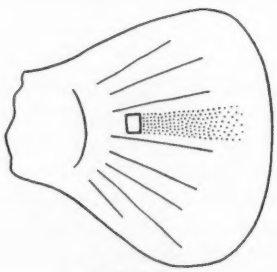

FIGURE 4

Figure 3.—A faded band in the tail of a killifish within which a new short cut has been made. This cut has induced the formation of a new small band within the larger one showing that the severed nerve-fibers of the original band are still active.

Figure 4.—A band in the tail of a killifish produced by cutting a hole instead of making merely a slit.

act in a positive way and over a long period of time on their appropriate effectors. This conclusion, moreover, is supported by experiments with adrenalin. If a small dose of adrenalin is injected into a killifish with a well-marked caudal band, the fish quickly becomes very light in tint and the dark caudal band fades completely. After 4 to 5 hours the effect of the adrenalin wears off and the fish gradually assumes its slightly darker tint. At the same time and without any further stimulation the dark band returns in its entirety. The original dose of adrenalin carried in the blood of the fish must have acted directly on the melanophores and in its characteristic way by inducing a concentration of the pigment. As this hormone disappeared, the dispersing nerves, whose action had been overridden by the adrenalin, must have begun to reassert themselves and the dark band consequently reappeared. This at least seems to be the most

plausible explanation of the conditions herein described and rests on the idea of continuous nervous activity even to the extent of the recovery of the band.

How the band recovers is an interesting question. The positive action of the nerve-fibres whereby the band is excited is believed to be due to the discharge from the activating nerve terminals of a neurohumor which when it reaches the melanophores induces them to disperse their pigment. Is the band after excitation maintained by a relatively large amount of locally held neurohumor or do the nerve-fibres after severance continue to produce this substance but in gradually diminishing amounts till they cease completely? This question may be tested by applying a cold-block, as already described, to the middle of a fully developed dark band. If the band is maintained by an initial and excessive discharge of neurohumor stored for the time being in the tissues, the distal half of the band after the application of the block ought to show no change. If, however, the band is maintained by a more or less continuous flow of impulses from the cut to the distally located melanophores, on the interruption of this flow by the block the distal part of the band ought to fade. As a matter of fact when this test is applied the distal half of the band does fade and fade unequivocally (Fig. 5). This condition is best seen in bands on the tail of the killifish which have about reached their maximum tint rather than in freshly excited bands. From the outcome of these tests the conclusion is again warranted that severed melanophore nerves are for a long time thereafter continuously active.

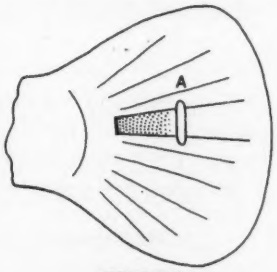

FIGURE 5

A fully formed band in the tail of a killifish to which a cold block (*A*) had been applied with the result that the distal half of the band faded in about a quarter to half an hour after the application of the block.

The nerve-fibres that are concerned with the melanophore responses in fishes have long been known to be of autonomic origin and those having to do with the dispersion of pigment are presumably parasympathetic in their source. Such fibres are non-medullated and to this condition might be ascribed their apparently exceptional peculiarities, for the general belief of neuro-physiologists in the inactivity of nerves separated from their centers is based upon the study of medullated elements. It must be admitted, however, that the nerve-fibres concerned with pain, fibres that are known to be medullated, may show more or less continuous functional activity not unlike that just described for the melanophore nerves. Moreover, by means of oscillograph methods Adrian (1930)

has identified post-operative discharges from mammalian medullated nerves and more recently Hoagland (1933) has recorded similar conditions in the lateral-line nerves of fishes. I am, therefore, led to conclude that probably many nerves, medullated as well as non-medullated, may remain more or less continuously active for long periods, days in fact, after their severance from their centers. This conclusion is put forward notwithstanding the fact that many neuro-physiologists hold a contrary opinion.

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*THE CENTRAL NERVOUS MECHANISM FOR EMOTIONAL RESPONSES: II. A TECHNIQUE FOR DESTROYING THE DEEPER NUCLEAR REGIONS WITHIN THE CEREBRUM WITH A MINIMAL DESTRUCTION OF THE INTERVENING CORTEX**

BY CLARENCE W. BROWN AND FRANKLIN M. HENRY

UNIVERSITY OF CALIFORNIA

Communicated April 14, 1934

Previous studies on the neural bases of emotional responses have indicated that certain nuclear regions in the thalamus are functionally significant in the rage response whenever these regions are released from the control of the cerebral hemispheres (Bard,[1] de Barenne[3] and Weed[7]). In these investigations acute decorticate or decerebrate preparations were used. The areas isolated for study were large and rather poorly defined. The intense responses involved in self protection, and usually termed rage responses, dominated the behavior of the animals and prevented the appearance of other types of emotional reactions.

In reviewing these studies a number of questions have arisen concerning the neural bases of emotional responses. First, would the functions of these deeper nuclei be different when the restraining influence of the cortical centers are removed than when these centers are intact? In order to test this a technique must be used which will allow for the destruction of the deeper nuclei with a minimal destruction of the overlying cortex. Second, are the neural bases for emotional responses localized within a definite, small nuclear region within the mesencephalon or diencephalon, or a number of these regions? To answer this question a method must be devised

for varying the amount of destruction within rather wide limits and for adequately controlling the locus of the destruction. Third, are *all* emotional responses innervated from the same region within the thalamus or midbrain? Reactions other than those called out in the rage response should be carefully studied. To do this animals with intact hemispheres should be used in order to restrain, to some degree, the intense rage reactions. Different nuclear regions in the mesencephalon and diencephalon should then be investigated. Fourth, would chronic preparations, from which many test reactions could be obtained, manifest responses varying in kind or intensity from those responses characteristically observed in acute animals? The solution of this problem demands the use of a technique and method which will cause the least possible shock to the animal and which will allow it to recover quickly and completely in order that postoperative experimentation can be conducted over a period of at least a month.

The present paper describes a technique for obtaining controlled destruction within the deeper nuclei of the cerebrum with a minimal destruction of the intervening cortex. It is a direct outgrowth of a number of studies† which have been made under the direction of the senior author to devise a method for investigating the functions of the deeper nuclei when the cortical areas are relatively undisturbed (Brown[2]). In these investigations the available techniques of destruction by injection of chemicals, mechanical use of the scalpel and coagulation by the electric cautery were tested. They were discarded on two counts. First, in no instance was it possible to destroy the underlying tissues without the involvement of relatively large amounts of cortex. Second, it was impossible to achieve a high degree of control over the locus and amount of the region being destroyed.

Preliminary experiments had shown that a method of producing destruction by localized application of radio frequency current offered considerable promise of success. A long series of cut-and-try experiments was therefore begun in which an attempt was made to devise a high frequency current generator which would furnish a variably-controlled current of suitable strength and frequency to accomplish any desired amount of destruction and which could be conducted, by means of an insulated wire electrode, into the deeper tissues with a minimal destruction of the intervening cortex. Two desiderata were always kept in mind: (1) control over the amount of destruction, and (2) control over the locus of destruction. Because of the number of factors determining the destructive capacity of high frequency current the realization of the first objective came only at the end of much experimentation. At one time or another the effects of the following factors were studied: (1) amperage and voltage, and (2) duration of the current, (3) the amount of electrode exposed, (4) the suit-

ability of various kinds of electrode insulators, (5) the vascularity and nuclear nature of the tissue.

As would be expected, some of these factors contributed more than others to the destructive effect of the current. The effects of the first two were not constant for all ranges, so it was necessary to determine the range in each which would give the desired variation in amount of destruction. A further complicating factor came from the tendency of the tissue to char when the voltage was too high. This charring was found to depend particularly upon the relationship between the voltage and the duration of the current, and to vary considerably at different ranges. It was necessary to eliminate all charring in the experiments on the functions of the deeper nuclei because the charred tissue often adhered to the electrode and on being removed tended to tear the surrounding cortical tissue.

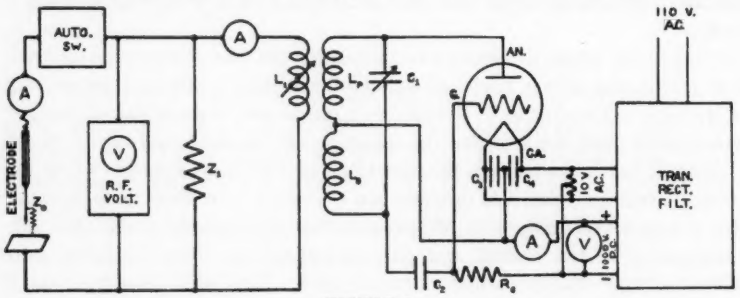

FIGURE 1

Schematic diagram of the high frequency unit. See text for explanation of symbols.

L_1 — 0.5 μh.	C_1 — 0.0005 μfd.	R_g — 10,000 ohms
L_p — 6.4 μh.	C_2 — 0.0003 μfd.	Z_1 — 10 ohms
L_g — 1.08 μh.	C_{3-4} — 0.002 μfd.	V. Tube — Type 211

The high frequency currents were generated by a vacuum tube oscillator (see Fig. 1), using the well-known Hartley circuit. The apparatus was designed in accordance with accepted radio engineering practice. Several conditions influencing the stability of the oscillator received particular attention (Prince[6]). The circulating volt-amperes in the circuit L_pL_g—C_1, was held considerably in excess of 4π times the power withdrawn from the oscillator, by using the proper value of C_1, and adjusting the mutual induction between L_p and L_1. The output voltage was adjusted by varying the anode voltage.

The frequency used was 3×10^6 cycles, which is well above the range of electrical stimulation, and furthermore is high enough to pass directly through the tissue cells (McClendon[5]). It was determined experimentally that the potential applied to the electrode had to be kept below about 20

volts if consistent results were to be secured. The impedance Z_0 of the electrode in situ, including such tissues as were in the current path, was found to be of the order of 1000 ohms at the frequency used, hence only about 0.4 watt of power was required to produce the tissue destruction.

The voltage at which charring and irregular destruction began to occur was found to be quite critical. Naturally the impedance of the electrode circuit varied considerably and erratically if the critical voltage was exceeded, a condition which was accentuated due to the fact that the output voltage of a radio frequency oscillator tends to vary directly with the impedance of the load. This effect was almost completely eliminated by using a radio frequency oscillator large enough to produce 40 watts of high frequency power, and dissipating most of this energy in a 10-ohm reservoir circuit Z_1.

Tissue destruction is produced by the action of the current, which, under certain conditions, is determined by the voltage across the electrodes. It was found that measurement and control of this voltage was of more value than the measurement of the electrode current. A vacuum tube voltmeter was used, the circuits of which were designed according to a method described by Lubke.[4] The error of this meter was 1% for comparative measurements, although its absolute accuracy was somewhat less.

Destruction in the cortical tissues through which the electrode passes is a function of the size of the electrode, the type of the electrode point and the presence or absence of sticking or charring of the tissue at the tip. Sticking and charring were prevented by the selection of the proper combinations of voltage and duration. In a study of the functional significance of subcortical mechanisms in learning,‡ a voltage of 16 with a duration of from 2 to $2^1/_2$ minutes has proved very satisfactory in producing experimental lesions. In no instance where this combination has been used has there been any indication of charring. Using this voltage and varying the duration of current between the limits of 0 and $2^1/_2$ minutes it was possible to produce subcortical destruction varying from the amount produced mechanically by the electrode to a volume of approximately 4.5 cu. mm.

The limiting factor in the case of electrode size was the rigidity of the wire. The most serviceable electrode proved to be a nickel silver wire 0.010 of an inch in diameter. This was given two coatings of bakelite varnish which increased the diameter to 0.014 inch and greatly added to the rigidity. The varnish was removed at one end and the wire ground to a point. Great care was exercised to get as smooth a surface as possible. With this type of tip the electrode tended to push aside the tissue as it was inserted and the immediate mechanical destruction around the electrode was actually less than the diameter of the coated wire. The diameter of the mechanical injury produced in nervous tissue by the shank of the elec-

trode was 166 micra. Allowing 20% for shrinkage of the brain during the histological preparation (which is a generous allowance as the tissue was prepared by a method which reduced any change in size to a minimum) the destruction amounts to 199 micra. The actual diameter of the electrode was 355 or 56 micra greater than the mechanical injury. This finding supports the contention that the sharpened tip pushes aside much of the tissue as it is inserted and that the actual destruction of cells is very small. It will be remembered that one of the requirements of the surgical procedure to be used in a successful study of the functions of the deeper nuclei was a minimal destruction of the overlying cortical tissues. This has been achieved in the use of the high frequency current.

It was apparent from the trial experiments that to obtain lesions of similar locus on opposite sides of the midline, and to produce definite variations in locus from one animal to another, it was necessary to use a modified micro-manipulator for inserting the electrode into the tissue. A wide range of variation in three dimensions was required. A device meeting these requirements is being constructed and will be described in another paper.

All of the experiments thus far conducted indicate that the high frequency unit provides a very satisfactory means for accomplishing the destruction of tissue in a small controlled area within the interior of a tissue mass. The unit provides a means whereby the functions of the deeper nuclei of the cerebrum may be investigated while they are under the normal restraining influence of the cortical centers. It provides a precise control over the locus and amount of destruction so that it is possible to study the individual nuclei of the diencephalic and mesencephalic regions. With the rage responses held in check by the cortical centers it now becomes possible to investigate the neural bases of other types of emotional responses. Because of the slight amount of tissue destroyed, the shock effects are not extreme, and it is very easy to keep the animals alive. Records on the behavior of the animals can then be made over a period of a number of weeks after full recovery from surgical shock and the other immediate effects of the operation.

Although primarily designed for studying the neural bases of emotional reactions the high frequency unit should prove serviceable in solving other anatomical and physiological problems in which a small controlled surgical insult is required. At the present time the architectonics of the basal ganglia are not fully known. The reason for this is not difficult to find when it is remembered that the picture of subcortical destruction is always complicated by the degeneration incident to cortical destruction. There is especial need for further studies on the nervous patterns within the thalamic region. The unit should prove serviceable in studying the physiology of small organs such as the corpora lutea, the separate lobes of the

pituitary gland, etc. The unit is now being used in studying the effects of controlled lesions in the subcortex upon the ability (1) to learn a maze, (2) to form the brightness discrimination habit, (3) to discriminate between inclined planes varying in degrees of steepness and (4) to discriminate certain visual patterns.

[1] Bard, P., "A Diencephalic Mechanism for the Expression of Rage with Special Reference to the Sympathetic Nervous System," *Amer. Jour. Physiol.*, **84**, 490–515 (April, 1928).

[2] Brown, C. W., "The Central Nervous Mechanism for Emotional Responses: I. Some Limitations of Previous Investigations with Suggestions for Further Experimental Work," *Jour. Comp. Psychol.*, **14**, 365–385 (Dec., 1932).

[3] Dusser de Barenne, J. G., "Recherches experimentales sur les fonctions du systeme nerveux central, faites en particulier sur deux chats dont le neopallium a ete enleve," *Arch. neerl. de physiol.*, **4**, 31–123 (1920).

[4] Lubke, H., "Vacuum Tube Voltmeter Design," *Proc. Inst. Rad. Engineers*, **17**, 864 (May, 1929).

[5] McClendon, J. F., "Colloidal Properties of the Surface of the Living Cell, II," *Amer. Jour. Physiol.*, **82**, 525 (Nov., 1927).

[6] Prince, D. C., "Vacuum Tubes as Power Oscillators," *Proc. Inst. Rad. Engineers*, **11**, p. 275 (June), p. 405 (August), p. 527 (Oct.), 1923.

[7] Weed, L. H., "The Reaction of Kittens after Decerebration," *Amer. Jour. Physiol.*, **43**, 131–157 (April, 1929).

* This is the second of a series of experimental investigations on the central nervous mechanism for emotional responses. A preliminary statement of the problem is to be found in reference 2 of the bibliography. The construction of the apparatus herein described was made possible by a grant-in-aid from the NATIONAL RESEARCH COUNCIL.

† A preliminary description of one of these studies is given in a Master's Thesis by Tecla Von Osten, University of California Library.

‡ A study is now in progress by the senior author and E. Ghiselli in which rats are subjected to bilateral lesions in various regions of the subcortex and then tested on problems involving discriminatory ability in the sensory fields of vision, audition, olfaction and kinesthesis.

RAIDING AND OTHER OUTSTANDING PHENOMENA IN THE BEHAVIOR OF ARMY ANTS[1]

BY T. C. SCHNEIRLA

DEPARTMENT OF PSYCHOLOGY, WASHINGTON SQUARE COLLEGE, NEW YORK UNIVERSITY

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Ants of the genus *Eciton* are characterized by their raiding expeditions, and by their temporary colony clusters or "bivouacs." In the matter of raiding, species in which the advance is formed by a large mass of ants may be termed the "swarm raiders," since their behavior is to be broadly distinguished from that of other species, the "column raiders," in which the raiding is marked by relatively small advance groups that head narrow columns.

The behavior of *Eicton hamatum*, the type species of the genus and a representative *column raiding species*, has been described.[2] In the raiding of this species a small group of ants, the "pushing party," heads the advance into new territory. The membership of a pushing party constantly changes, due to the fact that each newcomer first meanders excitedly forward, and leaves the group shortly after having made a few hesitant advances. As the ants move forward in this relay manner, the direction of progress is partly determined by the momentum of newcomers and partly by topographical features which (1) furnish guiding tactual stimuli (e.g., a course along the top of a log), or which (2) may split the pushing party, the result being a division of the group (e.g., some of the ants follow the edge of a buttressed root while the others turn to one side of the root).

Behind the advance group, ants move in a narrow column which follows a route first chemically saturated by the excited members of the pushing party. When the ants are very active in raiding, and the advance group is wider than the usual few centimeters, the frequent division of this body gives rise to numerous branching and anastomosing trails. In time, and as the supply of booty is depleted in a given area, the ants desert most of the trails there, and usually they continue to follow one trail that leads into a new raiding zone further on. In this manner the typical fan-shaped complex of moving ant columns is moved forward, and there remains behind a constantly lengthening serpentine route which is closely followed by ants passing up to enter the advanced raiding zone or returning booty-laden toward the bivouac. After a period of active raiding the *E. hamatum* colony will have formed one system or more of the type sketched in figure 1. The part played by tactual sensitivity in the matter has been suggested, and the chemical factor is of basic importance in the formation, the following and the extinction of trails. For instance, ants passing outward on the principal trail are able to respond to chemical differences that exist

between alternative routes at encountered trail junctions. One observes that newcomers turn onto a branch trail in numbers directly after the first booty-carriers have surged across the junction in passing toward the colony site.

Eciton vagans conducts similar raids from the colony bivouac site, which in that species is located beneath the surface of the ground. Although the consolidation columns that connect the raiding front with the bivouac mainly follow a subterranean course, the advanced part of the raid forms on the surface a system of branches and anastomosing columns very similar to that of *E. hamatum*. Also, the behavior of ants in the pushing parties does not differ greatly in the two species. The *E. vagans* raiders emerge upon the surface (e.g., from a crack in the ground or from some insect's tunnel), and if amounts of booty are found the raiding front is extended as in *E. hamatum* forays, with the disappearance of branch trails occurring in much the same manner. If little booty is taken, the ants withdraw along their principal trail into the ground, and may soon emerge at a point some meters removed to repeat the process there.

The Swarm Raiders.—Eciton burchelli, although similar in appearance to *E. hamatum*, is a much more excitable and belligerent ant, and its spectacular forays involve masses of raiders that clear insect life rather completely from terrain over which they pass.[3] In early morning the aroused workers spread out over the ground, and as the numbers increase the ants gradually cease to mill about and begin to move away from the colony site. The principal direction of progress is influenced by the manner in which newcomers pass into the body, and also by the general topography. As the forming swarm moves forward it occupies a wider front, and a meter or two in the rear the ants are so reduced in numbers that they form anastomosing columns on which the raiders scurry about erratically at first. The columns of this network (Fig. 2), left behind as the raiding front is advanced, become more distinctly formed in time, and their number is reduced. The result is that a few meters behind the raiding swarm the "consolidation system" dwindles into a single surviving column. The "consolidation trail," which is followed by ants that run between raiding front and bivouac, is a characteristic feature of all *Eciton* raids. Its development from the original raiding complex depends mainly upon the traffic in different parts of the raiding system and especially upon what quantities of booty are carried back through this or that part of the consolidation system, as well as upon the accessibility of alternative routes and the ease with which they may be followed by the ants.

The raiding of *E. burchelli* often involves swarms more than five meters in width. Larger bodies are likely to divide into two raiding systems; but rarely are more than two systems formed. The ants on the front and flanks of the swarm usually advance rather irregularly, especially when

passing through dense vegetation; and loose wide columns frequently push out from the main body. The ants on such columns advance rapidly and "draw" more raiders after them—a phenomenon based upon tactual sensi-

FIGURE 1

FIGURE 2

Figure 1. One raiding system of a nomad *Eciton hamatum* colony, two hours after the beginning of the foray. "A," the beginning of one system of trails; "B," the bivouac cluster beneath a log; "C," the point at which the second system of trails begins to branch from its consolidation trail.

Figure 2. The raiding system of a statary *Eciton burchelli* colony, three hours after the beginning of the foray. "A" marks the right flank of the raiding swarm, in which the ants are closely concentrated at present; "B" marks the left flank, in the course of a "wheeling" movement; "C," marks the consolidation column, which connects the raiding front with the bivouac, at "D."

tivity—if they chance upon some canalizing feature which permits rapid progress, and the advance of the main body may be definitely influenced if the column's pushing party chances upon quantities of booty.

Typically, the advance of the swarm involves alternate wheeling ("flank-

ing") movements; e.g., as the body swings toward the right, (1) the left flank rapidly spreads forward and is "drawn" inward and somewhat to the right, since (2) the right flank is greatly concentrated at the time and serves as pivot (see Fig. 2). Then the right flank spreads rapidly forward, while the left flank of the swarm slows in its progress and soon becomes concentrated in its turn. This alternate flanking movement of the raiding swarm is mainly responsible for the meandering course that is taken by the eventual consolidation trail. The phenomenon occurs principally as a result of the manner in which contact among individuals limits and directionalizes the spreading of a mass of eccentrically running workers over an area; but the process is complicated by other factors as well, and varies greatly at times.[4]

Eciton praedator likewise is a "swarm raider." Colonies of this species bivouac in subterranean places, but the pattern of raiding behavior is very similar to that of *E. burchelli*. From the place of emergence a mass of the small ants, often more than three meters in width, advances on the surface in the meandering manner described above, but the advance varies more frequently in its general direction. The swarm of small ants leaves behind it an anastomosis of wide and loosely formed columns which converges into a few consolidation columns in which the ants pass beneath leaves to points in the rear. As in *E. burchelli* raids, here and there behind the mass there remains a concentration of ants engaged in "mopping up," busily capturing booty that escaped the principal swarm.

The Bivouac-Change.—As is well known, the *Eciton* colony makes its nest by gathering into a cluster, and the site of this "bivouac" changes from time to time. In *E. hamatum* and *E. burchelli* colonies one may ascertain two conditions of general activity, which alternate according to the condition of the brood.[5] The colony remains bivouacked in a given place, in the "statary condition," first when the eggs are present and during the period that precedes the development of the eggs into food-consuming larvae, and secondly during the period of about three weeks when the young are enclosed in cocoons. In contrast, the colony removes to a new bivouac site each evening (is in the "nomad condition") during the time the larvae are naked and are consuming food, and again, after the brood has been hatched.

Colonies in the statary condition are less active, and raid much less vigorously than when in the nomad condition. To illustrate the difference, the raiding swarm of a nomad *E. burchelli* colony usually divides into separate masses, while that of the statary colony rarely does so; the nomad *E. hamatum* colony usually forms two or three separate raiding systems during its forays, while the statary colony forms but one system (Fig. 1) and on occasional days does not raid at all.

Eciton raids typically show two peaks of activity, one during the morning and the other during the afternoon. These bursts of activity appear to be

related to meteorological changes, as does the first arousal of raiding early in the morning. In nomad *E. hamatum* colonies the second high point in activity precedes a general movement of the colony which begins later in the afternoon and is completed during the first half of the night, as a rule. If three raiding systems have been formed during the day, with three corresponding principal trails leading from the bivouac, the ants respond increasingly to that route on which the most booty is being carried back, and smaller numbers venture forth on the other trails as time goes on. At length, the streaming outward of greater numbers of ants on the eventual bivouac-change route makes difficult the return of booty-carriers on this trail from the advanced raiding front, and these ants are virtually forced to deposit their burdens in piles that form near the places of greatest confusion—i.e., at trail junctions. Consequently, late in the afternoon the raiders are observed mainly returning to the bivouac on the other raiding systems, while on the "popular" system cachés of booty are forming at trail junctions in the advanced raiding area, and few ants succeed in carrying their burdens back to the bivouac. The outward-streaming column of ants at first stimulates raiding by giving it new recruits, but later interferes with it mainly by virtue of the fact that the direction in which ants turn at trail junctions is more and more completely determined by the pressure of the general movement. Usually, in the case of *E. hamatum*, the new bivouac cluster is located between 100–200 meters from the previous site. The *E. burchelli* movement typically gets under way much later in the day, and often follows the principal route of a raiding system established during the afternoon peak of activity.

Other Colony Activities.—The social life of the ants in and about the relatively thin-walled bivouac cluster presents an intriguing problem. Beebe, for instance, has described the manner in which the *E. burchelli* colony behaves at the time the larvae spin their cocoons.[6] An equally interesting event is the opening of cocoons when the pupae are mature. Judging from experiments, it is rarely that an *E. burchelli* or *E. hamatum* callow escapes unassisted from its case. Rather, the adults respond to the movements of the enclosed individual, pick it up from among the cocoons heaped within wall pockets of the cluster, and carry this cocoon to the outside of the wall. At first ants catch hold of the ends of the cocoon, but as other workers are excited by the stir and join the group a tear is started sooner or later in the middle of the case by some worker's mandibles (and possibly by the tarsal hooks of the enclosed moving callow), and the case is eventually ripped open as this tear is enlarged. The enclosed individual struggles out as the workers lick and tug upon her body, and is soon carried into the interior of the cluster, while the empty case falls to the ground.

The opening of cocoons usually requires from two to four days, since the pupae differ somewhat in the time at which they attain maturity. The

larger workers and majors stir before the smaller workers do, in these two species at least, and are therefore removed from their cases before many of the minors begin to darken and to move within their cocoons.

The number of individuals in the Eciton brood is large. In a captured *E. hamatum* colony of about 30,000 workers, there were counted 32,479 cocoons. However, *Eciton* colonies cannot increase indefinitely in size, since at cocoon-opening time, after many new individuals have emerged, a portion of the colony moves from the statary site along a raiding route and forms an independent colony.[7] This would suggest a rapid increase in the number of colonies, and indeed army ant colonies are plentiful; but prolific reproduction is probably compensated by decimation of numbers and possibly by extinction of entire colonies during the dry season.

[1] The NATIONAL RESEARCH COUNCIL, through its Committee on Grants-in-Aid, has supported an investigation of *Eciton* behavior, carried out during the summers of 1932 and 1933 on Barro Colorado Island in the Canal Zone. The first portion of the work has been reported in the *Jour. Comp. Psychol.*, April (1933).

[2] A list of references to the literature on the Ecitons will be found in the paper cited in note 1, and in a paper by W. M. Wheeler, *Proc. Am. Acad. Arts Sci.*, **56**, 291–328 (1921).

[3] These raids have been described by different writers, notably by Bates (Bates, H., 1863, *The Naturalist on the River Amazons*) and by Belt (Belt, T., 1874, *The Naturalist in Nicaragua*). Any ground or lower tree animal life that is accessible or that cannot escape is torn into pieces and carried back to the colony bivouac. Scorpions and tarantulas are frequently captured, but the only vertebrate that I have seen killed was a small Coral snake that happened to be resting in a cul-de-sac under a tree root. Not infrequently, lizards dart in and out among the masses of ants, snatching up insects flushed out by the *Ecitons*, as do the Ant Birds from their perches near the ground. The Ant Birds attend the swarm raids almost exclusively, since the manner in which the column raiders cover territory limits their booty largely to the larvae and pupae of insects. To capture such an object from the ant holding it would necessitate swallowing both morsel and *Eciton* carrier, and these birds seldom eat army ants.

[4] A detailed report on this and related matters in the behavior of representative *Eciton* species is to be published during the latter part of the present year.

[5] W. Müller (Kosmos, 1886), from his study of an *Eciton burchelli* colony, was first to suggest the possibility of a relationship between the food-consuming ability of the *Eciton* colony, as affected by the presence of active larvae, and the general activity of the group.

[6] Beebe, W., "The Home Town of the Army Ants," *Atlantic Monthly*, April, 514–522 (1917).

[7] Wheeler (op. cit., note 2) found two "...evidently recently emerged females" in an *E. burchelli* colony he examined during the latter part of the rainy season in British Guiana, and suggested that new *Eciton* colonies may be formed through the fission of older colonies. The division which I have observed to take place in *E. hamatum* and *E. burchelli* colonies on a number of occasions during the first two months of the Panama rainy season indicates that such is the case. These instances of fission must have been based upon females of the previous season, since it is a notable fact that no queen or male forms were found among the brood of several colonies examined during the months of June and July.